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Effects of Pressure on the Respiration of Vertically Migrating Crustaceans[1]

By William G. Pearcy and Lawrence F. Small

Department of Oceanography
Oregon State University, Corvallis, Ore., U.S.A.

ABSTRACT

The rates of oxygen consumption of *Euphausia pacifica* Hansen, *Thysanoëssa spinifera* Holmes, and *Sergestes similis* Hansen at 1 atm were compared with the rates at 16–50 atm. Differences were not significant, indicating little effect of pressure on respiratory rate. Apparently temperature has more influence than pressure on the respiration of these species during their diel vertical migrations.

INTRODUCTION

Many marine animals are sensitive to hydrostatic pressure. Small changes in pressure of 1 atm or less often elicit changes in swimming behavior or activity (see Knight-Jones and Morgan, 1966, for review). However, little is known about the effect of pressure on the metabolism of aquatic organisms, especially deepsea or vertically migrating animals that normally experience extreme pressures or extreme diel changes in pressure. Recent studies have indicated the influence of temperature on the respiration of vertically migrating animals (McLaren, 1963; Lasker, 1966; Small and Hebard, 1967), but we need to know if hydrostatic pressure also modifies respiration before the results of such studies can be extrapolated to the respiration of animals in nature.

This study was undertaken to learn if there is an effect of hydrostatic pressure on the oxygen consumption of three species of common pelagic crustaceans off Oregon: *Euphausia pacifica* Hansen, *Thysanoëssa spinifera* Holmes, and *Sergestes similis* Hansen. *Euphausia pacifica* is an oceanic euphausiid known to migrate vertically several hundred meters within the upper 500 m (Brinton, 1962, 1967). *Thysanoëssa spinifera* is a neritic euphausiid which undertakes a limited diel migration (Brinton, 1962; M. Alton, personal communication). *Sergestes similis* is a sergestid shrimp which migrates within the upper 500 m (Pearcy and Forss, 1966).

METHODS

Animals for the experiments were captured in nets towed from the R/V *Yaquina* during various seasons of the year, between 9 and 120 km off the central Oregon coast. Short duration net tows were made within the upper 100 m after darkness, usually with 1-m diam plankton nets, although on

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several occasions an Isaacs–Kidd midwater trawl was towed slowly near the surface to collect large euphausiids and shrimp.

The contents of the codend buckets of the nets were immediately emptied into plastic containers filled with fresh sea water. Individual euphausiids and sergestids were removed with large-bore plastic pipettes or nylon gauze funnels and maintained in 1- or 2-liter containers filled with unfiltered sea water in the dark at 10 C. If the animals were not used within a few days after capture, the water was changed periodically and the animals were fed *Artemia* nauplii or mixed phytoplankton cultures.

All experiments were conducted in constant temperature rooms, generally at 10 C. Several experiments were carried out aboard ship immediately after the animals were collected, but most were done ashore. Animals were removed from their holding containers and rapidly transferred with coarse nylon funnels or stainless mesh wire spatulas, which did not retain small plankton or detritus, into 65- or 130-ml glass B.O.D. bottles. The water in these bottles had been membrane filtered (0.8 μ nominal pore size), or filtered through No. 12 nylon bolting cloth for shipboard experiments, and air-equilibrated at the cold-room temperature.

Bottles, each containing one animal, then were stoppered with either glass or finely tapered neoprene stoppers. Neoprene-stoppered bottles were placed inside a pressure chamber, 135 mm inside diam by 450 mm deep. Glass-stoppered bottles were placed inside a darkened container and held at 1 atm pressure. During each experiment several bottles without animals were included as controls both inside and outside the pressure chamber. We increased the pressure by using a hydraulic pump filled with glycerin and distilled water. A Heise temperature-compensated gauge indicated pressure in pounds per square inch above 1 atm. The pressure inside the chamber was increased in a step-wise fashion during the first 30–60 min of an experiment then maintained at constant pressure during the remainder of the experiment. Experiments lasted 16–24 hr, depending upon the size of the animals and the size of the bottles used. The pressures selected were equivalent to those at depths of 150–475 m in the ocean, a range that included the daytime depths of the oceanic animals studied.

At the end of an experiment, the pressure was rapidly released and the condition of the animals was noted in each bottle. Occasionally dead or moribund animals were found; oxygen consumption for these was not included in the results. A Winkler procedure was used to determine dissolved oxygen (Strickland and Parsons, 1965). Animals were dried to constant weight in a dessicator or drying oven. Average oxygen consumption during the experiment, expressed as μliter (mg dry wt)$^{-1}$ hr^{-1}, was calculated from the difference in dissolved oxygen between the bottles incubated together with and without animals.

RESULTS AND DISCUSSION

The results of 19 experiments on 282 animals are summarized in Table I. Increased pressure had no apparent effect on the oxygen consumption of

TABLE I. Comparison of respiration of *Euphausia pacifica*, *Thysanoëssa spinifera*, and *Sergestes similis* subjected to 1 atm and higher pressures.

Date	Temp (*C*)	Outside pressure chamber (1 atm)			Inside pressure chamber			
		No. animals	μliter $O_2 mg^{-1} hr^{-1}$		Pressure (*atm*)	No. animals	μliter $O_2 mg^{-1} hr^{-1}$	
			Mean	Range			Mean	Range
Euphausia pacifica								
July 16, 1965	10	2	1.39	1.37–1.41	32	3	1.57	1.22–2.09
Dec. 8, 1965	10	5	1.32	1.14–1.47	32	3	1.17	1.11–1.28
Mar. 10, 1966	10	3	2.38	1.50–3.90	16	17	1.71	1.13–2.07
July 1, 1966	10	7	2.04	1.12–3.86	35	7	2.57	1.77–3.32
July 19, 1966	10	5	1.79	1.19–2.61	35	4	2.28	1.56–3.06
Nov. 21, 1966	7–10	5	1.10	0.80–1.79	35	8	1.74	1.10–2.96
Dec. 20, 1966	10	11	1.66	0.89–2.32	35	13	1.15	0.65–1.82
Jan. 23, 1967	10	7	1.24	0.98–1.94	50	4	1.19	0.90–1.52
Pooled estimate		45	1.61±0.63[a]			59	1.64±0.64[a]	
Thysanoëssa spinifera								
Apr. 12, 1966	4–8	8	2.12	1.64–3.79	16–18	6	2.05	1.67–2.31
Apr. 12, 1966[b,c]	4–8	8	1.96	1.19–3.03	18	8	1.72	1.22–2.32
July 19, 1966	10	5	1.69	1.38–2.17	35	6	1.96	1.35–2.98
July 20, 1966	10	10	2.81	1.91–3.85	35	12	2.97	1.61–3.96
July 20, 1966	10	10	2.39	1.58–4.43	35	10	2.95	1.32–4.23
Aug. 4, 1966	10	15	2.05	1.46–2.85	35	13	1.78	1.16–2.12
Pooled estimate		56	2.21±069[a]			55	2.29±0.88[a]	
Sergestes similis								
Mar. 1, 1966	10	2	0.95	0.63–1.28	35	2	1.12	0.89–1.34
Jan. 18, 1967	10	11	0.77	0.37–1.11	50–52	9	0.83	0.70–1.06
Jan. 20, 1967	10	3	1.66	1.34–1.91	50	5	1.83	0.96–3.75
Feb. 18, 1967	10	10	1.50	0.61–2.08	50	11	1.26	0.69–1.74
Feb. 17, 1967	10	10	1.42	1.05–1.76	35	5	1.25	1.16–1.32
Pooled estimate		36	1.23±0.44[a]			32	1.21±0.55[a]	

[a]One standard deviation of the estimate.
[b]Conducted aboard ship within 12 hr of capture.
[c]Guts filled with phytoplankton.

the three species of Crustacea studied. For each species there was no significant difference between pooled mean respiration of control animals and animals at high pressure. Of particular interest is the fact that *T. spinifera*, subjected to pressures much greater than normally experienced, showed no change in oxygen consumption with increased pressure. Considering all species together, animals subjected to high hydrostatic pressures had slightly higher average oxygen consumption than control animals in 10 experiments, but the reverse was true in the remaining 9 experiments. In every experiment the ranges of oxygen consumption of animals inside the pressure chamber overlapped the respiratory ranges of control animals. Statistical significance was not computed for individual experiments because of the relatively small number of animals per experiment.

The lack of correlation between respiration and pressure does not mean that the animals are insensitive to changes in hydrostatic pressure; it only indicates that no large changes in oxygen consumption accompany high pressures. These experiments were conducted at pressures thought to approximate those experienced by the species in nature (except for *T. spinifera*). If abnormally high pressures had been used, tolerance limits might have been approached, causing behavioral changes or death which would have been reflected in oxygen consumption (Regnard, 1891, as cited in Johnson et al., 1954; Fontaine, 1930; Napora, 1964).

Only a few other reports are known on effects of pressure on respiration of vertically migrating euphausiids or shrimp. In a series of experiments on six species of euphausiids from the tropical Atlantic and Indian Oceans and one species from the North Atlantic Ocean at different temperatures and pressures, Teal and Carey (1967) found that pressure affected respiration in only two species. One of these species, *Thysanopoda monacantha*, was mesopelagic and did not come to within 150 m of the surface at night. Respiration of *T. monacantha* was approximately constant with depth, indicating that temperature-induced decrease in respiration was offset by pressure-induced increase. Napora (1964), working with the mesopelagic shrimp *Systellaspis debilis*, also found that pressure effects on respiration essentially compensated for temperature effects. Teal and Carey suggested that constant metabolism throughout the normal migratory range of mesopelagic Crustacea might be the rule.

The other species affected by pressure in the experiments of Teal and Carey was *Meganyctiphanes norvegica*, a species endemic to the North Atlantic. The respiration of *M. norvegica* was slightly decreased by pressure of 100 atm, so that metabolism at a depth equivalent of about 1000 m was somewhat lower than if there had been no pressure effect. However, pressure effects were not significant at pressures corresponding to depths of 0–500 m, a range which probably includes the maximum depth of *M. norvegica*.

The other five species of euphausiids investigated by Teal and Carey showed a lack of metabolic response to pressure changes encountered during normal migrations. This result was similar to that we achieved with two

euphausiids and a shrimp from the North Pacific. Within normal depth ranges it appears that temperature is more important than pressure in modifying respiratory rates of species that are found in epipelagic waters at night.

The three species in this paper had significantly different oxygen consumption rates. After pooling data from inside and outside the pressure chamber, averages and standard deviations were computed for each species as follows: *T. spinifera*: 2.25 μliter O_2 mg^{-1} hr^{-1} ($\pm$0.79); *E. pacifica*: 1.64 μliter O_2 mg^{-1} hr^{-1} ($\pm$0.63); *S. similis*: 1.22 μliter O_2 mg^{-1} hr^{-1} ($\pm$0.50). The average value of *E. pacifica* was higher than values reported for this species at 10 C (by using manometry) by Lasker (1960, 1966), Small et al. (1966), and Small and Hebard (1967); however, one standard deviation $\pm$ the mean included the mean values reported in these other papers. Using Winkler determinations, Paranjape (1967) reported a low average consumption for *E. pacifica* (0.79 μliter mg^{-1} hr^{-1}) at 10 C which he believed was due to the unusually low oxygen concentrations in the fjord where he collected his animals.

Thysanoëssa spinifera had a higher respiratory rate than *E. pacifica*. This result was in contrast to that of Small et al. (1966), who found no difference in weight-specific respiration between the two species. Season of the year might have been a factor, however. They made their comparisons with animals collected in the winter and the early spring. *Thysanoëssa spinifera* specimens in this study were collected in the summer, except for some collected in April (and these had intestinal tracts filled with phytoplankton, a condition which could have increased respiration). Small and Hebard (1967) measured no seasonal differences in respiration of *E. pacifica*, but this species characteristically undertakes rather extensive vertical migrations and is subjected daily to a relatively wide temperature range. *Thysanoëssa spinifera*, on the other hand, migrates much shorter vertical distances, and might become acclimated to either relatively high or relatively low temperatures in the summer, depending on the intensity of upwelling. Strong upwelling brings relatively cold, saline water to the surface layers along the Oregon coast in summer, but there are years in which upwelling is weak and surface waters remain warm through the summer. Acclimation to different temperatures in different seasons could effect changes in respiratory rates of *T. spinifera*. The molting and reproductive physiology, condition, and acclimation history of the animals also might have contributed to differences in measured respiration in the various studies.

Mean weight-specific respiration of *S. similis* was less than that of the two species of euphausiids, regardless of pressure level. Respiration of *S. similis* was measured only during winter months, however. A contributing factor also might have been the different weight ranges of the three species. Decreased weight-specific respiration with increased animal weight has been documented for many crustaceans (see Wolvekamp and Waterman, 1960). In our studies, *S. similis* weighed between 5 and 173 mg dry weight (mean = 74 mg), which was significantly greater than either *E. pacifica* (range = 1–13

mg, mean = 5 mg) or *T. spinifera* (range = 2–59 mg, mean = 21 mg). However, when weight-specific respiratory rates were plotted against animal weight, variability was great and no definite trends were noted. Small and Hebard (1967) and Paranjape (1967) concluded that weight-specific respiration of *E. pacifica* was not a function of weight.

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Inheritance of Muscle Proteins in Hybrids Between the Redside Shiner (*Richardsonius balteatus*) and the Peamouth Chub (*Mylocheilus caurinum*)[1]

By Nevin Aspinwall

Department of Zoology
University of British Columbia, Vancouver, B.C.

AND

H. Tsuyuki

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

ABSTRACT

Selected muscle proteins were examined in artificially reared redside shiners, *Richardsonius balteatus*, peamouth chubs, *Mylocheilus caurinum*, and in their F_1 and backcross hybrids to determine if they would be useful in identifying suspected natural hybrids. In reference to the proteins studied, *Mylocheilus* possesses a single zone designated "3"; *Richardsonius* also possesses a single zone designated "1," but of slower mobility. Artificially reared F_1 hybrids possess, in addition to both parental zones, a unique zone designated "2" which was assumed to be composed of components of zones 1 and 3. Some naturally occurring individuals suspected of being hybrids on the basis of morphological characters possessed a pattern identical to that of the experimentally produced F_1 hybrid (zones 1, 2, 3). Other suspected hybrids possessed either zones 1 and 2 or zones 2 and 3. These types of pattern corresponded to some of those produced by backcrosses to *Richardsonius* and *Mylocheilus*, respectively.

The presence of both parental protein zones in the F_1 hybrid indicates that each polypeptide can be synthesized when only half the alleles of each parent is present. This characteristic of codominance can be useful in identifying suspected hybrid offspring of parents having different protein zones.

Evidence is presented which supports the hypothesis that the proteins in question are genetically controlled by two loci, A and B, each of which exists in two allelic forms.

INTRODUCTION

Characteristically, hybrid fishes have been recognized by their morphological intermediacy with respect to the putative parents (Hubbs, 1955). This approach, developed by the noted ichthyologist Dr Carl L. Hubbs and his colleagues, has generally proved satisfactory. However, several weaknesses of the method seem apparent. Firstly, the problem of recognizing a hybrid becomes increasingly difficult as the morphological similarity between two hybridizing individuals increases. Thus, an underestimate of the frequency of hybridization may result in those taxonomic groups whose members are

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morphologically quite similar. Secondly, morphological intermediacy, per se, does not necessarily indicate hybrid origin. One species may be morphologically intermediate to two others merely as the result of fortuitous selection. Usually, other corroborating evidence such as frequency of occurrence, fertility, and the sex ratio of a suspected hybrid is needed.

The present communication describes a method which was found useful in identifying suspected hybrids between the redside shiner *Richardsonius balteatus*, and the peamouth chub *Mylocheilus caurinum*, on the basis of the electrophoretic properties of their muscle proteins.

A model is also proposed for the genetic control of these proteins.

MATERIALS AND METHODS

Suspected interspecific hybrids between the peamouth chub and redside shiner were collected from Stave Lake, British Columbia, during the summers of 1966 and 1967. In addition, F_1 and backcross hybrids were experimentally produced and reared at the experimental station on Stave Lake. The progeny of such crosses were maintained on an identical diet regimen of *Artemia* nauplii, plankton, and frozen brine shrimp until they reached a sufficient size (35 mm) to permit electrophoretic analysis. Prior to analysis, all progeny and the wild fish used as parents in the 1967 crosses were frozen. Unfortunately, the carcasses of the parents used in the 1966 crosses were preserved in 10% formalin which denatured their muscle proteins.

Samples of muscle were homogenized in low ionic strength (0.055 M) phosphate buffer, *p*H 8.5, for approximately 15 sec until the homogenate possessed a creamy texture. The ratio of muscle to phosphate buffer was 1:2, respectively. The homogenate was then centrifuged for 15 min at 37,000 $\times$ *g* and the supernate collected. Starch-gel electrophoresis of the supernate was conducted at 180 v for 2.25 hr using a micro method previously described (Tsuyuki et al., 1966). Protein zones were stained with amido black 10B as before.

RESULTS

Muscle Proteins of Natural Populations

At the concentrations of the muscle protein extracts tested in these experiments, several characteristic major and minor zones were present. In this communication, discussions are confined to those major protein zones designated 1, 2, and 3 which are diagnostic and significant in the analysis of hybridization between *Mylocheilus* and *Richardsonius* (Fig. 1). Representative muscle protein electropherograms of naturally occurring *Mylocheilus* (Fig. 1A), *Richardsonius* (Fig. 1E), and the suspected hybrids (Fig. 1B–D) are presented. Specimens of *Mylocheilus* from within and outside (Alouette, Ruby, Hatzic, and Babine lakes and Stave River) the hybridization area possess zone 3, whereas *Richardsonius* is characterized by zone 1, a protein of considerably slower anodal mobility. This clearly defined difference in

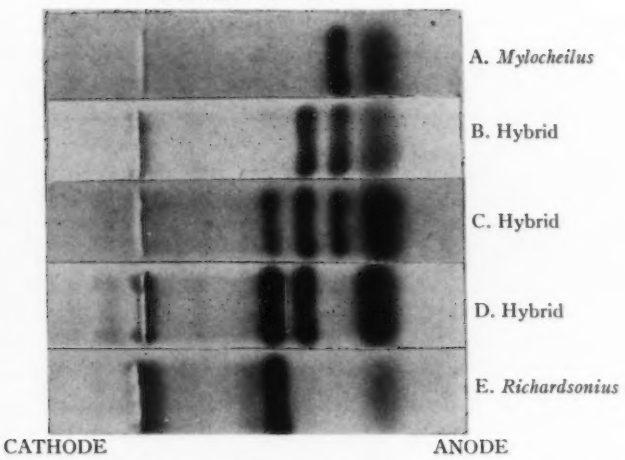

FIG. 1. Starch-gel electropherograms of muscle proteins from *Mylocheilus caurinum*, *Richardsonius balteatus*, and their hybrids. Samples are from Stave Lake, British Columbia.

mobilities of the proteins of the two parental species was largely instrumental in the selection of this group of proteins as a diagnostic tool for the recognition of hybrids. All of the suspected hybrids in Fig. 1B–D share a unique intermediate protein zone designated "2," as well as one or the other or both parental zones 1 and 3.

Muscle Proteins of Artificially Reared Fish

Crosses of *Mylocheilus* × *Mylocheilus* and *Richardsonius* × *Richardsonius* resulted in progeny with the protein patterns characteristic of the respective parents (Table I). The F_1 progeny resulting from reciprocal crosses of

Table I. Muscle proteins of parents and progeny of crosses made in 1966 and 1967.

Protein patterns																							
Parents																							
Type of cross		Zone no.						Progeny (no. of individuals) Zone no.															
		Female			Male			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Female	Male	1	2	3	1	2	3			+		+	+		+	+	+	+	+			+	
Mylocheilus	*Mylocheilus*			+			+	40															
Mylocheilus	"Hybrid"			+	+	+	+	4			5			3									
"Hybrid"	*Mylocheilus*	+	+	+			+	6			22			14									
Mylocheilus	*Richardsonius*			+	+									40									
Richardsonius	*Mylocheilus*	+					+							40									
Richardsonius	"Hybrid"	+			+	+	+							1								1	
Richardsonius	"Hybrid"	[a]			[a]									4			5						
"Hybrid"	*Richardsonius*	[a]			[a]									7			9			5			
Richardsonius	*Richardsonius*	+			+															33			

[a]Parental patterns from 1966 crosses not available.

Mylocheilus and *Richardsonius* produced a single electrophoretic pattern possessing zones 1–3 and corresponding to the pattern found in some suspected natural hybrids (Fig. 1C). Reciprocal backcrosses of hybrids to *Mylocheilus* (BCM) produced offspring with three types of protein pattern: (*a*) the parental *Mylocheilus* pattern (zone 3); (*b*) the pattern possessing zones 1, 2, and 3; and (*c*) a new pattern possessing only zones 2 and 3 and corresponding to that of Fig. 1B. Reciprocal backcrosses of hybrids to *Richardsonius* also produced progeny with three types of protein pattern: (*a*) the parental *Richardsonius* pattern (zone 1); (*b*) the pattern possessing zones 1, 2, and 3; and (*c*) a new pattern possessing only zones 1 and 2 and corresponding to that of Fig. 1D.

DISCUSSION

The study of the protein patterns of progeny produced by experimental hybridization clarifies some of the problems relating to the history of hybridization in natural populations of *Mylocheilus* and *Richardsonius*. For example, individuals possessing only zone 1 may either be pure *Richardsonius* or hybrid backcrosses to *Richardsonius* whereas similarly those possessing zone 3 may either be pure *Mylocheilus* or hybrid backcrosses to *Mylocheilus*. The three-zoned pattern (Fig. 1C) may be possessed by the backcrosses of hybrids to either parental species or the F_1 hybrid. However, roughly 50% of the backcrosses of hybrids to either parental species produce offspring with a unique protein pattern (Fig. 1B and D). Thus the naturally occurring hybrids shown in Fig. 1 are not all F_1 hybrids. The pattern of Fig. 1C may represent a backcross to either species or the F_1 hybrid. The hybrids represented in Fig. 1B and D, represent offspring of backcrosses of hybrids to *Mylocheilus* and *Richardsonius*, respectively. It should be mentioned that a very high proportion of experimentally produced F_2 hybrids (hybrid $\times$ hybrid) were found to be inviable. Thus, the probability of these existing in nature is very low.

An interesting aspect of the inheritance of the parental proteins is that the loci controlling their synthesis are codominant; that is, both parental zones 1 and 3 are synthesized in the F_1 hybrid. Since codominance appears to be a general phenomenon when the primary gene product is detectable, it may prove useful in identifying F_1 hybrids of parents possessing unique proteins which are readily detectable by techniques such as electrophoresis which can be adapted to a study of large numbers of specimens. The advantage of this type of analysis over that which utilizes morphological characters is that primary genetic contributions from both parents can be detected separately in a single individual. In contrast, morphological characters of a hybrid usually resemble neither parent but are intermediate to them. Codominance has been found in proteins in hybrids between members of the families Salmonidae (Tsuyuki and Roberts, 1965; Hochachka, 1966), Centrarchidae (Manwell et al., 1963), Gasterosteidae (Hagen, 1967), Cyprinidae (present communication), and in many other organisms.

A genetic model explaining the inheritance of protein zones 1 and 3 in *Richardsonius* and *Mylocheilus*, respectively, is depicted in Fig. 2. The model states that zones 1 and 3 are each controlled by two loci, "A" and "B," each of which exists in two allelic forms (A and A′; B and B′). *Mylocheilus* is depicted as possessing the genotype, $\frac{A}{A}\frac{B}{B}$, and *Richardsonius* $\frac{A'}{A'}\frac{B'}{B'}$. Although the model depicts loci A and B as being on separate chromosomes, they may, in fact, be located on the same chromosome but at least 50 map units apart.

Assumptions which are implicit in the model are: (1) proteins detected in zones 1 and 3 are controlled by genes present in both parental species; (2) the products of the two loci complex to form a dimer which is unique to each parental species; (3) protein zone 2 represents a hybrid dimer composed

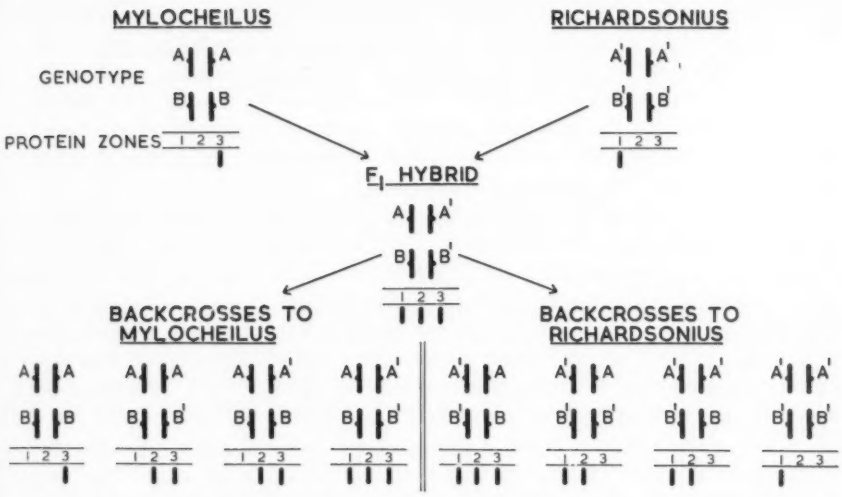

FIG. 2. A genetic model for the inheritance of muscle proteins 1 and 3 in *Richardsonius* and *Mylocheilus*.

of a polypeptide contributed from each of the parental species; (4) the polypeptides of protein zone 1 are electrophoretically indistinguishable as are those of protein zone 3.

The true breeding quality of artificial parental crosses is evidence for homozygosity of the postulated alleles A and B in *Mylocheilus* and A′ and B′ in *Richardsonius*. Further evidence is the lack of variation in these protein zones in either species outside the area of hybridization (130 *Mylocheilus*, 65 *Richardsonius* examined). The distribution of protein patterns in hybrid backcrosses to both species supports the idea of dual gene control. For example, in backcrosses to *Mylocheilus* (Fig. 2) a new type of pattern is found in addition to the expected parental (zone 3) and F_1 (zones 1, 2, 3) patterns. This new pattern contains zones 2 and 3 but not zone 1. If zone 2 is composed of subunits of zones 1 and 3, its presence indicates that only half of the components required to produce zone 1 are present. The half of the components of zone 1 which is present in this phenotype combine with the components of zone 3 to produce zone 2. Thus, only half of the genetic complement of one parental species is present in these phenotypes; the other half has segregated to another gamete. In backcrosses to *Richardsonius* a similar situation occurs in which the new pattern possesses only zones 1 and 2, indicating that only half of the components needed to produce zone 3 are present. In a similar fashion to the BCM two-zoned progeny, the components of zone 3 which are present combine with those of zone 1 to form zone 2.

If the two-gene model is correct, the backcross progeny to both species should possess one-zoned, two-zoned, and three-zoned patterns in a ratio

of 1:2:1, respectively. This hypothesis was not rejected for either BCM progeny ($X^2 = 1.81$) or BCR progeny ($X^2 = 2.75$; rejection at $p = 0.05$, 2 df = 5.99).

Although sex data was not gathered on experimentally produced F_1 progeny, there is no reason to suspect that the sex ratio is drastically different from 1:1. Evidence from natural hybrids supports this contention. Since a single protein pattern was obtained for both reciprocals of F_1 crosses, it appears that the "A" and "B" loci are not sex-linked.

The above model does not explain how the genes' products aggregate to produce the proteins under discussion. The data presented in Fig. 1 lend some support to the idea that the four alelles (A, A′, B, B′) each produce subunits which assemble into dimers in a partially random fashion. Assuming that each protein requires subunits produced by both loci, A and B, zone 2 should appear in the three-zoned pattern in a double concentration relative to zones 1 and 3 since it can be formed in two ways, A′B and AB′, whereas zone 1 and 3 can be formed only by A′ B′ and AB, respectively (see Fig. 2). In the BCM two-zoned pattern, zones 2 and 3 appear to be in equal concentrations (Fig. 1B) as is expected since each of these zones can be formed in two ways; zone 2 by AB′ and AB′ or A′B and A′B, and zone 3 by AB and AB. A similar situation exists in the two-zoned BCR progeny.

ACKNOWLEDGMENTS

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Comparative Electrophoretic Patterns of Lactate Dehydrogenase in Three Species of Trout[1, 2, 3]

By G. R. Bouck[4] and R. C. Ball

Department of Fisheries and Wildlife
Michigan State University, East Lansing, Mich., U.S.A.

ABSTRACT

The isozyme patterns of lactate dehydrogenase (LDH) were examined in three species of trout using electrophoresis in polyacrylamide gel slabs. Each pattern was species specific and contained 15 isozymes of LDH, but a hybrid trout had at least 27 LDH isozymes. Tissue specificity of these isozymes was low except for skeletal muscle. These results suggest that LDH isozyme patterns could be used for taxonomic and diagnostic purposes.

INTRODUCTION

The potential uses of lactate dehydrogenase (LDH) in trout research include the identification of different species, as well as clinical evaluation of physiological condition. Our interests mainly concern the latter because rainbow trout (*Salmo gairdneri*) have become the standard cold-water test fish in many pollution investigations. Unfortunately, the current literature reveals very little information concerning rainbow trout LDH and does not produce a concensus. Therefore, we undertook and now report studies concerning: (1) the species specificity of LDH patterns; (2) the total number of LDH isozymes (fractions); (3) some effects of hybridization in (1) and (2) above; and (4) the distribution of LDH isozymes in various tissues.

Factors that reduce the integrity of cells (i.e., hypoxia, histotoxicity) allow damaged cells to leak various enzymes including LDH. This leakage elevates the total LDH activity of plasma or serum in higher vertebrates (Hess, 1963), and if this also applies to fish, one could detect pollution-induced cellular damage by analyzing micro blood samples for LDH. Furthermore, the determination of total plasma or serum LDH is sensitive, rapid, and simple to perform. Another potentially valuable aspect of LDH is that it exists in several molecular forms that can indicate the site of damage (Lawrence, 1964). This is possible because of the following considerations.

LDH is a complex protein unit consisting of four subunit protein molecules that are bound together by four molecules of nicotinamide adenine dinucleotide (NAD) (Klontz, 1967). In most organisms, there are only two different types of LDH subunits and the total combinations of these two subunits,

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[4]Present address: Pacific Northwest Water Laboratory (FWPCA), Corvallis, Oregon 97330.

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taken four at a time, yield five enzymatically active but different forms of LDH called isozymes (Markert, 1963). Electrophoresis can separate the total LDH into its various isozymes. Fortunately, certain LDH isozymes are found mainly in specific tissues, hence, LDH isozymes can often identify discrete damage in higher vertebrates (Lawrence, 1964). Again, this might be valuable in studying tissue damage caused by pollutants.

Electrophoretic analyses have previously provided a biochemical basis for species identification and, in some cases, allowed the identification of racial groups (Tsuyuki et al., 1966; Tsuyuki and Roberts, 1966; Tsuyuki et al., 1964a, b; Nyman, 1965; Hewitt et al., 1963; Lillevik and Schloemer, 1961). This taxonomy is possible because carefully controlled electrophoretic analyses separate proteins into fractions that have species specific mobility. But each fraction is still a heterogeneous mixture of proteins, and factors that alter the physio-chemical properties of proteins can also alter their electrophoretic mobility. Although the use of complex protein patterns may be desired in some cases, it seems preferable both to limit these investigations to fewer proteins, and to increase sensitivity of protein identification by utilizing the biochemical function of a protein rather than its available dye binding sites.

The number of LDH isozymes in trout has been studied, but these investigations have not produced a concensus of opinion. Using disc electrophoresis, Goldberg (1965) found nine LDH isozymes in brook trout (*Salvelinus fontinalis*). His results indicated both that three different monomers of LDH were involved and that the total number of LDH isozymes would be 15. Morrison and Wright (1966) reported 15 LDH isozymes in trout. But in a later study of brook trout, Goldberg (1966) usually did not find more than six LDH isozymes and he concluded that his previous results were indicative of a single highly inbred population. Goldberg (1965) also reported that rainbow trout had only three or at most four LDH isozymes. On the other hand, Bouck (1965) reported more than nine LDH isozymes in rainbow trout, and believed that even more LDH isozymes were present in this species. In the latter case, a different electrophoretic method was used and will be described in the following methods section.

One can infer from the above studies that LDH in trout is more complex than it is in most organisms and that the mobility of the isozymes may allow the identification of different species and racial groups as well as specific tissues.

METHODS AND MATERIALS

Most of the fish for these studies were supplied by the Michigan Department of Conservation. The study included four each of the following species: (1) brook trout (*Salvelinus fontinalis*); (2) lake trout (*Salvelinus namaycush*); and (3) rainbow trout (*Salmo gairdneri*). In addition to these, the Michigan Conservation Department also supplied three albino brook trout and four brook–brown hybrids (called tiger trout). Lastly, the sample included four rainbow trout (Kamloops variety) that were obtained from a private hatchery located at Soap Lake, Washington.

Each fish was anesthetized with 1:50,000 M.S. 222 before tissues were excised. Tissue extracts were prepared immediately by the following procedure. One part tissue (either skeletal muscle or heart plus brain) and two parts ice-cold 0.25 M sucrose solution were blended in an ice-cooled glass mortar. The resulting slurries were centrifuged at 4 C for 85 min at 30,000 × *g*. A 1-ml portion of each supernate was decanted and a tiny crystal of bromphenol blue was added to each, thus allowing visual inspection of the samples in the gel slots. Each sample was electrophoresed in its own slot. Human serum was treated in the same manner; human albumin binds this dye and the dye-bound albumin served as a visible reference during electrophoresis. After incubation was complete, the bromphenol blue had usually diffused out of the gel. However, normal serum contains recognizable amounts of LDH activity and human LDH-3 served as a reference point after incubation.

The LDH patterns of various tissues were investigated in rainbow trout and these tissues were prepared by the following procedure. After a small volume of blood was drawn via the exposed heart, approximately 0.1 ml of 10% heparin in physiologic saline (Stokes, MS, 1963) was injected to inhibit coagulation. Shortly thereafter, the ventral aorta was canulated and the fish was perfused with approximately three times its volume of physiological saline (ice cold). Blood and saline were drained via an incision in the heart. After this perfusion the gills were colored white, the liver was light brown, and both the kidney and spleen were dull gray; this indicated almost complete blood removal. But this degree of blood removal was achieved only once in many attempts, hence, the use of tissues from a single specimen was necessary for a priori reason. Extracts of these tissues were prepared as described above.

Electrophoresis was conducted using an apparatus described by Raymond (1964). Both the 5% acrylamide gel and the buffer were made according to Peacock et al. (1965) except that smaller amounts of the stock solution were made and were discarded after 1 week. A 10-μliter portion of human serum or tissue extract was inserted into individual gel slots after a 20-min pre-electrophoresis to clean the gel. Samples were migrated 8 hr at 200 v across the bridge; tap water at about 12 C was circulated through the cooling plates of the cell both before and during electrophoresis. At the end of the run, human albumin was located at the extreme anodal end of the gel slab. None of the fish extracts retained bromphenol blue in amounts that were visible.

Immediately after electrophoresis, the gel slab was placed in a plastic box of approximately the same size, containing freshly prepared incubation medium, modified somewhat from that of Markert and Urspring (1962). The incubation medium consisted of 4 ml of 0.04 M KCN, 4 ml of 0.5 M lactate, 12 ml of 1.0 M 2-amino-2-(hydroxymethyl)-1,3-propanediol, 80 mg NAD, 3 mg phenozine methosulfate, and 50 mg nitroblue tetrazolium (NBT). The *p*H was adjusted to 7.3 and the final volume was diluted to 125 ml. The *p*H was checked every half-hour and readjusted to 7.3.

The essence of this test is that LDH functions to convert lactate to pyruvate; this reaction is then coupled to a second reaction that converts NBT

(colorless) to reduced NBT (purple colored and insoluble in water). Hence, color is produced via the enzyme, and this reaction can be allowed to continue until the locations of the enzyme are evident. These reactions are discussed in greater detail by Markert and Urspring (1962).

The resulting position of the LDH isozymes was recorded by measuring the distance that each isozyme had migrated. Attempts to photograph these patterns were generally unsatisfactory for two reasons: (1) the resulting photographs lost much desired detail and emphasized artifacts that were not visible to the investigators; and (2) the high enzyme activity of certain fractions caused excessive color production that often obliterated adjacent isozymes.

In preliminary studies, the incubation proceeded at room temperature until 1 hr after the last LDH band had become clearly evident. This established that the optimum incubation period for this technic was at least 5 hr. When lactate was omitted from the medium, no color production resulted unless the *p*H was above *p*H 8.0. The latter resulted in diffuse color production both in the gel and in the medium, but this did not form bands. The possibility that these procedures had produced anomalous LDH fractions was investigated and rejected because no such effect was noted in human LDH that was treated in the same manner. Likewise, the possibility that the samples had become cross-contaminated either by diffusion between the sample slots, or by diffusion of the separated fractions during the long incubation was also investigated and rejected.

RESULTS

Species Specificity

The resulting isozyme patterns of trout LDH are shown in Fig. 1 and are depicted in Fig. 2. Each species had an LDH pattern that was specific for that species. Although no variation was observed in the LDH patterns for each of these species, a larger sample size may reveal subsequent variations. However, Nyman (1965) examined the esterase patterns of 200 Atlantic salmon (*Salmo salar*) and reported no variations in their patterns.

Rainbow trout from Michigan (Wisconsin variety) and from Washington (Kamloops variety) did not have any apparent differences in their LDH patterns. However, albino brook trout had lower amounts of enzyme activity in certain muscle LDH isozymes than did normal brook trout.

While the LDH patterns were sufficiently different to allow species identification, the patterns also show certain similarities. The electrophoretic mobilities of LDH from brook and lake trout were quite similar, but LDH isozymes from rainbow trout were much more closely spaced (see Fig. 1 and 2). These similarities closely parallel the chromosome counts (2 N = 84, 84, and 60 respectively, Wright, 1955).

Number of Isozymes

The tissue extracts of brook, lake, and rainbow trout each had a total of exactly 15 isozymes as judged by the production of colored bands. These

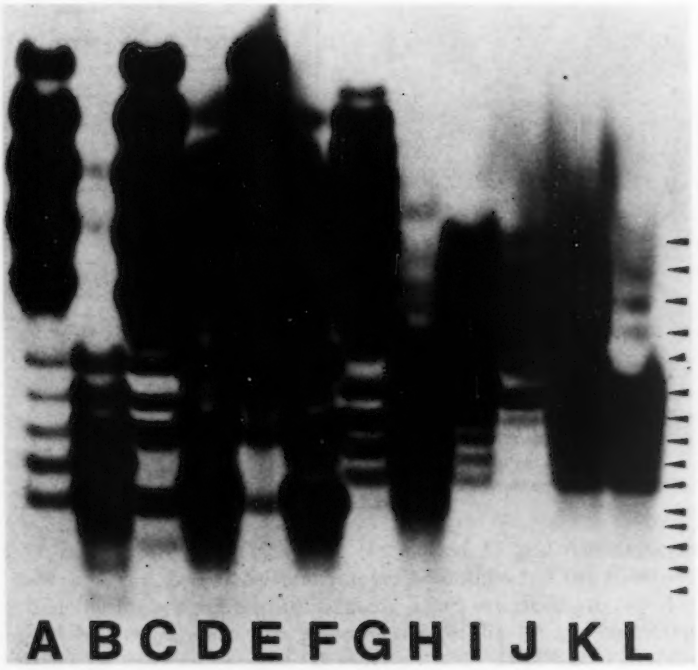

FIG. 1. Lactate dehydrogenase (LDH) patterns in trout. Lake trout skeletal muscle (A) and heart–brain (B); normal brook trout skeletal muscle (C) and heart–brain (D); albino brook trout skeletal muscle (E) and heart–brain (F); brook–brown hybrid skeletal muscle (G) and heart–brain (H); rainbow trout skeletal muscle (I, 2×; J, 1×); skeletal muscle–heart–brain (K); and heart–brain (L). Arrows along right margin indicate the positions of rainbow trout LDH isozymes. Not all the isozymes had developed bands at this point during the incubation period. Note differences between the relative LDH activity in normal and albino brook trout skeletal muscle.

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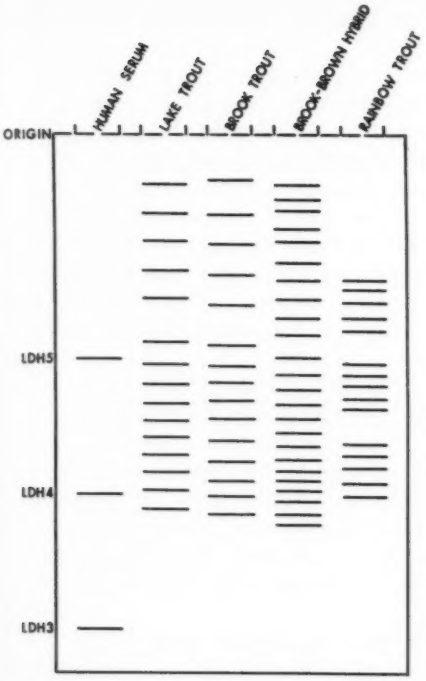

FIG. 2. Diagrammatic representation of lactate dehydrogenase (LDH) isozymes in man and trout. Human LDH 2 and LDH 1 had left the anode end of the gel during the long migration period. Lines indicate the positions of LDH isozymes, not their activity.

consisted of three systems of isozymes (1, 2, and 3) and each system had five isozymes (*a*, *b*, *c*, *d*, and *e*). System 1 never predominated any tissue extract, but these had the highest mobilities. System 2 had intermediate mobility and predominated heart–brain extract. System 3 had the lowest mobilities and predominated the activity in skeletal muscle.

EFFECTS OF HYBRIDIZATION

The total number of LDH isozymes in tissue extracts of brook–brown trout (F_1) hybrids were difficult to assess but showed essentially the same number of LDH isozyme systems as did the parent types. The outstanding difference in the hybrid specimens was that the total number of isozymes rose markedly within a given system. Also, a given isozyme system occupied essentially the same space but it contained so many isozymes that the total number could not be counted accurately. At least 27 were counted in the extracts of one hybrid specimen but only 25 were counted in the other specimens. These numbers must be viewed as representing minimum counts.

DISTRIBUTION OF ISOZYMES IN A FISH

The presence of the various LDH isozymes in the tissues of a single rainbow trout are depicted in Fig. 3. Hemolyzate contained only one isozyme (LDH,

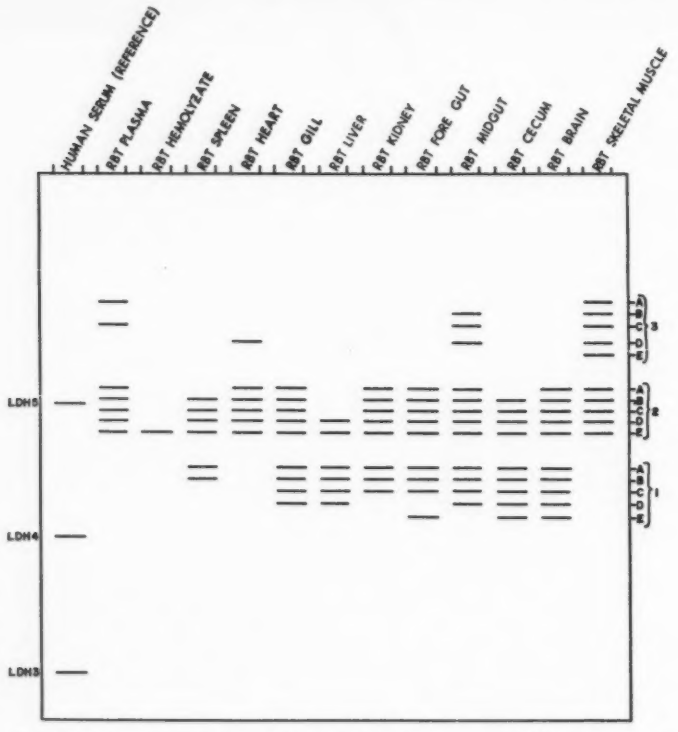

FIG. 3. Tissue specificity of lactate dehydrogenase (LDH) isozymes in rainbow trout. Includes only the isozymes that were clearly evident after an 8-hr incubation.

2*e*) and this was the only tissue that did not exhibit isozymes from at least two of the three isozyme systems.

The sample of midgut contained representatives of all three isozyme systems. Midgut contains pancreatic tissue dispersed among the pyloric ceca, and this may explain why it was the only sample to exhibit all three isozyme systems. However, it may be that every tissue contains all 15 isozymes, albeit that some isozymes may be present in very low amounts.

DISCUSSION

Based on these results, the LDH patterns of fish probably could be used to identify species, but these patterns were very similar between brook trout and lake trout. Furthermore, the LDH patterns did not indicate any differences between two races of rainbow trout. Although the combined use of other enzyme patterns might disclose greater species differences, or racial differences, such effort seems questionable in most instances because other

protein classes (eye lens, myogens, etc.) are either more specific or easier to utilize.

Possible exceptions to the above may exist when postmortem degradation has changed the myogen or other protein patterns, or when the amount of the sample is too limited to allow adequate staining by conventional technics. In the case of postmortem degradation, sufficient change may be imparted to the degraded proteins to cause a different-appearing protein pattern per se. Hence, these changes may alter the protein pattern, but may not affect the LDH pattern because only the unaltered molecules contribute to enzymatic activity. In the case of samples that are too dilute or otherwise insufficient, the sensitivity of the enzymatic color-producing reaction is many times more sensitive than conventional protein staining methods. For example, one can easily produce satisfactory LDH patterns from samples that are too dilute to exhibit any protein fractions after conventional staining technics.

The total number of LDH isozymes in a fish is undoubtedly under genetic control; however, the electrophoretic procedure can influence the apparent number of fractions. We consistently found 15 LDH isozymes in trout when electrophoresis was conducted in polyacrylamide gel slabs. Likewise, Morrison and Wright (1966) reported the same value when they used electrophoresis in starch gel slabs. But disc electrophoresis (method of Davis, 1964) failed to reveal 15 LDH isozymes both in our studies and in Goldberg's (1965, 1966) studies.

The classical number of LDH isozymes is five (one LDH system) and because trout display 15, it seems necessary to discuss the possibility of artifacts that might cause this outstanding exception. One possibility is that our electrophoretic procedure caused anomalous LDH isozymes. Certain chemicals such as B-mercaptoethanol, can do this (Fritz and Jacobson, 1963). We reject the artifact hypothesis for three reasons: (1) no such effect was produced to human LDH treated in the same manner; (2) tissue specificity of LDH patterns existed and would have been destroyed by such an effect; and (3) these results are in agreement with those derived by another electrophoretic method (Morrison and Wright, 1966).

On the other hand, there are several lines of evidence that support these results. Fifteen LDH isozymes have been reported before in genetic variants of man (Boyer et al., 1963) and in *Peromyscus* spp. (Shaw and Barto, 1963) and other counts exceeding 5 but less than 15 have been reported for other species (i.e., Zinkham et al., 1963; Morrison and Wright, 1966). In the case of trout, we consistently obtained 15 LDH isozymes in each of three species and we also obtained at least 27 LDH isozymes in hybrid trout.

Based on our experience, we can suggest why one might not obtain 15 LDH isozymes in trout. Undue storage or heating reduces the number of LDH isozymes and a low ambient temperature ($\leq$15 C) during electrophoresis seems essential. Decreased numbers of LDH isozymes also occur if serum is used for the analysis, if the electrophoretic migration is too short, or if the acrylamide–bisacrylamide stock solution deteriorates slightly. In the latter

cases, the isozyme systems may be resolved, but the individual isozymes are usually not resolved. On the other hand, a short incubation can produce equally aberrant results. This is because very high enzyme activity exists in only one LDH system per tissue, hence, short incubations rapidly develop five isozymes before the remaining isozymes are evident. In this case, one might easily assume that the first resulting five colored bands represented all the LDH present, especially when the classical number of LDH isozymes is five. However, if the incubation is continued, the remaining 10 isozymes will produce sufficient amounts of color to be clearly evident.

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Marking Fish with Stable Strontium[1]

By I. L. Ophel and J. M. Judd

Biology and Health Physics Division
Chalk River Nuclear Laboratories
Atomic Energy of Canada Limited, Chalk River, Ont.

ABSTRACT

Stable strontium is fed to fish in a Sr-rich, Ca-free synthetic food for a short period of time. Subsequent identification by flame emission photometry can be made using a single scale from a marked fish. One year after marking, scales from marked fish contain approximately 10 times as much Sr as those from control fish. There is no evidence of damage to fish due to their elevated Sr content.

INTRODUCTION

In fisheries management and fish biology there is a need for a method of marking large numbers of fish for later identification. Methods now in use (Lagler, 1952; Arnold, MS, 1965) include finclipping, metal or plastic tags, and the injection of chemicals or dyes. All of these procedures involve extensive handling and some mutilation of the fish. Immersion of fish in dyes produces short-lived marks (Arnold, 1965). Marking with radioisotopes (Scott, 1963) has disadvantages in public waters.

As an out-growth of experimental work on the metabolism of strontium by fish we have found it possible to mark fish permanently with stable strontium. The stable strontium is fed to the fish in a Sr-rich, Ca-free synthetic food for a short period. Subsequent identification can be made using a single scale from a marked fish.

An earlier experiment (Ophel and Judd, 1967) showed that goldfish (*Carassius auratus*) could accumulate, in 3 weeks, more than 3% Sr in the ash of bony tissue. Consequently a single scale from a small fish would contain about 2 μg Sr. Goldfish normally contain less than 0.1 μg Sr per scale. Since the analytical method (Judd and Coveart, 1965) for Sr used in our laboratory can measure less than 0.1 μg Sr, it seemed possible that a method for marking fish could be developed.

Feasibility depends largely on: (1) a long biological half-life of the deposited strontium in the scales; (2) absence of harmful effects on the fish; (3) rapid and positive identification of scales from marked fish.

EXPERIMENTAL PROCEDURES

Forty-eight goldfish, each weighing about 5 g, were divided randomly into two equal groups. They had previously been acclimated to the experimental water temperature (20 C) and to laboratory feeding. One group was

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fed ad libitum with a basic synthetic food (Ophel and Judd, 1967) to which was added 32 g of strontium lactate per 400 g of wet diet. The other (control) group received the same basic food but 30.8 g of calcium lactate was substituted for the strontium salt. Each diet thus contained 500 meq/kg wet weight of the appropriate alkaline-earth cation. The calcium lactate contained 2.9 μg Sr/mg Ca, which is within the range of ratios found in natural food (Ophel and Judd, 1968).

Both groups received these foods for 21 days. They were then transferred to running water and fed on commercial fish-food for the remainder of the experiment. The commercial food (Ophel and Judd, 1967) contained 5.3 μg Sr/mg Ca.

At intervals a single scale was taken from each fish and analysed for strontium and calcium. Scales were removed from the side of the fish just posterior to the operculum and near the lateral line. Analyses for both elements were made by flame emission photometry using a standard addition technique (Judd and Coveart, 1965).

Preparation of bony tissues for analysis in our laboratory is by wet-ashing with nitric acid and hydrogen peroxide. We compared several rapid methods of preparation with our method. Direct leaching of scales with 1 ml of 0.1 N HCl in 10% glycerol for 2.5 hr proved to be a satisfactory alternative (Table I), particularly as the solution could be aspirated in the flame photometer without further treatment. Glycerol was present in the leaching solution because it is required as a "releasing agent" in the analytical method. This method was used throughout the experiment.

TABLE I. Comparison of methods of scale preparation.

Method	Control fish		Marked fish	
	No.	μg Sr per scale ±SD	No.	μg Sr per scale ±SD
Standard (HNO_3 ashing)	5	0.11±0.06	5	1.89±0.94
Rapid (HCl leaching)	5	0.14±0.07	4	2.22±1.60

RESULTS AND DISCUSSION

Table II summarizes the results of the 1-year experiment. Initially, scales from the marked fish contained approximately 3 μg Sr per scale. This decreased quite rapidly to approximately 1.5 μg and then showed no detectable decrease for the remainder of the experiment. If the initial loss is regarded as due to a single rapid component then this fraction has a biological half-life of about 11 days. The biological half-life of the slow component cannot

TABLE II. Strontium content of scales after feeding fish synthetic foods.

Days[a]:	2	4	20	31	38	45	59	95	119	151	224	267	364
						μgrams Sr per scale (mean ± SD)							
Sr diet	3.1 ±1.0 $n=23$	3.2 ±1.2 $n=22$	2.5 ±1.0 $n=23$	2.1 ±0.7 $n=24$	1.8 ±0.5 $n=15$	1.8 ±0.7 $n=24$	1.6 ±0.8 $n=24$	1.5 ±0.6 $n=24$	1.6 ±0.5 $n=24$	1.8 ±0.8 $n=24$	1.5 ±0.6 $n=23$	1.7 ±0.7 $n=22$	1.5 ±0.5 $n=22$
Control diet	0.05 ±0.03 $n=8$	0.05 ±0.04 $n=16$				0.06 ±0.04 $n=8$				0.08 ±0.06 $n=24$			0.13 ±0.06 $n=24$

[a]Day 0 = last day of feeding synthetic foods.

be calculated from the data, but it is obviously many years — longer than the life span of most fish. Scales from the control fish initially contained 0.05 μg Sr. This amount increased as the fish (and scales) increased in size. However, at the end of a year, the control scales contained only 0.13 μg, less than 10% of that in scales from marked fish.

There was no evidence of damage to fish due to their elevated Sr content. All marked fish behaved normally and accepted food well during the experiment. Average weight of the 24 marked fish at the end of the year was 11.5 g compared with 11.1 g for the control fish. There was no evidence of disturbance in the calcium metabolism of the fish. Scales from both groups were analyzed for calcium three times during the experiment and the results were 35.3 ± 16.6 μg Ca per scale for the marked group and 35.7 ± 13.8 for the control group. MacDonald et al. (1951) have raised the bone ash content of Sr in rats to 10% before disturbances in metabolism were observed.

Reproducibility of Sr content was tested by taking two sets of scales from eight randomly selected marked fish. One set was found to contain 2.1 ± 0.9 μg Sr per scale and the other contained 2.1 ± 0.8 μg. Later, a further test was made by analysing 10 scales from a single fish. The mean Sr content

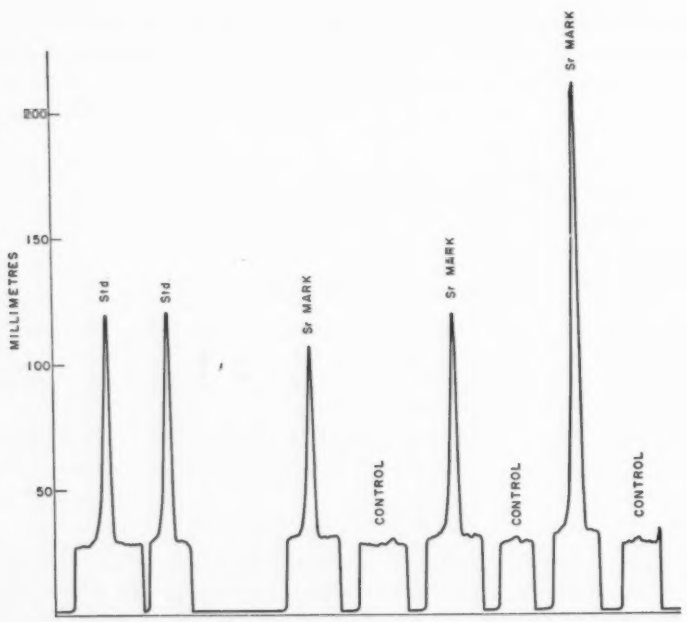

FIG. 1. Flame photometer recorder traces of single scales from three Sr-marked fish compared with three control fish one year after marking. Scales leached with 1 ml 0.1 N HCl in 10% glycerol. Traces of standard Sr solution (1.4 μg/ml), made under same instrumental conditions, shown on left.

per scale was 1.7 ± 0.65 μg, indicating a similar coefficient of variation between scales from the same fish to that between scales from different fish.

Identification of marked scales presented no difficulties throughout the experiment. Spectral scans were made of the flame emission over the 458–464 mμ wave-length region. The most sensitive Sr atomic resonance line is at 460.7 mμ. By adjusting the sensitivity of the flame photometer it is, in fact, possible to obtain recorder traces which show a peak height of 75 mm or more for scales from marked fish and no significant response for scales from control fish. Figure 1 shows such a trace where three scales from marked fish, one year after marking, are compared with three from control fish.

A number of modifications could be made if the Sr marking method was developed for field use. The feeding period could be shortened by increasing the strontium lactate content of the food. Several scales could be combined for analysis, instead of using a single scale as suggested here. For scaleless fish any small portion of bony tissue such as a spine or fin-ray could be used for analysis.

For marking hatchery-raised fish the method should need little adaptation. A similar synthetic food has been used by Wolf (1951) for dietary studies with hatchery trout. Marking wild fish would be difficult, but not impossible if young fish of a suitable size were trapped then held in live-cages and fed for a short time.

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Ultrastructure of the Striated Muscle of the Scallop (*Placopecten magellanicus*)[1,2]

By C. M. Morrison[3] and P. H. Odense

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

ABSTRACT

The striated adductor muscle of the scallop *Placopecten magellanicus* is described. The fusiform uninucleate cells contain one fibril which is divided into A and I zones, and has Z and H bands. The A zone is 1.9 μ long, and in this zone apart from the H band about eight actin filaments surround each myosin filament. A sarcoplasmic reticulum envelops the fibril. Tubules of this reticulum are mainly oriented longitudinally and swellings from dyads with the sarcolemma.

INTRODUCTION

The adductor muscle of monomyarian pelecypods is usually divided into a slow, opaque smooth muscle and a fast or translucent muscle. The structure of the fast muscle is variable. *Pinna attenuata*, *Atrina japonica*, and *Gryphaea angulata* have a smooth translucent muscle (Kawaguti and Ikemoto, 1959; Hanson and Lowy, 1959). The smooth muscle of *Gryphaea* has thick filaments of paramyosin. *Crassostrea angulata* has obliquely striated muscle with paramyosin filaments, and discrete dense bodies instead of a Z line (Hanson and Lowy, 1961). *Spondylus cruentus* has striated muscle with some oblique fibres (Kawaguti and Ikemoto, 1959). *Promantellum hirasei* (Kawaguti and Ikemoto, 1960) and the scallops *Pecten albicans* (Kawaguti and Ikemoto, 1958) and *Pecten yessoensis* (Samosudova and Slavskaya, 1961) have striated muscle. The scallop striated muscle consists of uninucleate cells with fibrils which have typical A and I zones, and H and Z lines. Kawaguti and Ikemoto (1958) described only one type of myofilament, which was 70 Å in diam in the A band and 50 Å in diam in the I band. Samosudova and Slavskaya (1961) resolved two types of filament, one 200–250 Å in diam, the other 50–75 Å in diam. Mattisson and Beechey (1966) showed one electron micrograph of the striated muscle of *Pecten maximus* in which a sarcotubular system was evident. Vesicles which form dyads with the sarcolemma are described in *Crassostrea angulata* (Hanson and Lowy, 1961), but otherwise there is no mention of a sarcoplasmic reticulum in the pelecypods we have mentioned. In our study of *Placopecten magellanicus* we attempt to clarify the description of the myofilaments and describe the sarcotubular system.

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[2]Based on a thesis submitted by C.M.M. in partial fulfillment of the requirements for the degree of Master of Science in the Department of Biology at Dalhousie University, Halifax, N.S.
[3]Formerly C. M. Bishop.

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METHODS

Scallops were obtained live from George's Bank and kept in the seawater tanks at the Halifax Laboratory until needed. All the following operations were carried out at 4 C until the stage of dehydration in 90% alcohol or acetone was reached, when the sample was allowed to come to room temperature. The fixatives used were 2 or 4% gluteraldehyde solutions and 2% osmium tetroxide solution. The fixatives were made up just before use in filtered seawater or 0.05 M phosphate buffer at *p*H 7.3, with 1% $CaCl_2$ added until the solution just failed to turn cloudy.

To obtain scallop striated muscle at rest length the muscle was exposed by sawing the scallop in half through both valves and the muscle. The muscle was obtained in a stretched state by keeping the valves apart with rubber bungs, and cutting away the tissues until the muscle was exposed. The muscle was either excised then fixed in gluteraldehyde fixative, or the fixative was dripped onto the exposed muscle until the fibrils no longer contracted when they were excised. In both cases the excised muscle was finely minced in gluteraldehyde fixative, and left in the fixative for 3 hr. The tissues were then washed in the solution used to make up the fixative for 2 hr, fixed in the 2% osmium fixative for 1½ hr, then briefly rinsed in distilled water. Tissues fixed using phosphate buffer solutions were dehydrated in a graded series of acetone–water solutions, finishing with 100% acetone, and embedded in Araldite. Those fixed using sea water were treated in the same fashion except that ethanol was used instead of acetone, and the samples were then passed through propylene oxide before they were embedded in Araldite. The blocks were cured for 3–5 days at 60 C. Gold, silver, and gray sections were cut with the LKB Ultratome, and picked up on grids which were coated with formvar stabilised with carbon. Sections were stained with 25% uranyl acetate in absolute methanol (Stempak and Ward, 1964) followed by Millonig's lead stain (Millonig, 1961), and examined in an Hitachi HS7S electron microscope.

Most of the scallops were kept in the tanks for several months, and used in December and January. Some were kept in the tanks for only a few days, and used in April and May.

OBSERVATIONS

Muscle fixed in December and January is shown in Fig. 1–7, and muscle fixed in April and May has the appearance shown in Fig. 8.

When muscle is excised from the scallop before fixing, the myofibrils are disoriented and the tubular systems are convoluted. The orientation is better when the muscle is fixed in situ, and this preparation is shown in the micrographs.

In light micrographs the muscle cell is seen as a narrow uninucleate body which tapers at both ends (Fig. 1A). The myofilaments usually form a compact striated fibril which extends the full length of the cell. The sarcomere length is 1.9 μ. A and I zones, and H and Z bands are evident in electron micrographs

(Fig. 1B, 2, and 3). No M or contraction bands have been seen. There are vesicular and tubular elements next to the sarcolemma, and there is little substance between the cells. Some mitochondria are present, which in material fixed using sea water appear long, narrow, and densely staining with closely packed cristae (Fig. 1B). By comparison, mitochondria fixed using phosphate buffer appear swollen (Fig. 2).

Thick and thin myofilaments are resolved at fairly high magnifications (Fig. 3). The thick and thin filaments overlap except for the H zone in the centre of the A band. There are connections between the thick and thin filaments except for this zone. The thick filaments are 165–175 Å in diam and the thin filaments are 25–35 Å in diam. The thin filaments appear to overlap and become thickened in the centre of the I zone to form a Z band.

A transverse section of the A band (Fig. 4A) shows that the thick filaments are arranged alternately in parallel rows, with about eight small filaments around each large one. The thick filaments have a less dense core, and sometimes have lateral branches. In the H zone (Fig. 4B) few thin filaments are present. The Z zone in transverse section (Fig. 4C) lacks the tetragonal arrangement of vertebrate muscle filaments (Knappeis and Carlsen, 1962). The filaments are thickened, and there are signs of lateral connections.

The sarcotubular system appears to consist of disjointed vesicles and tubules in preparations which used fixatives dissolved in phosphate buffer. However, those prepared with sea water show a continuous sarcoplasmic reticulum of vesicles and tubules (Fig. 5). The tubules are sometimes well oriented longitudinally in the cell (Fig. 6A), and have circular profiles in transverse sections (Fig. 6B). The vesicles often form dyads with the cell membrane (Fig. 3, 5, 6A, and 6B), and are a similar shape in longitudinal and transverse sections.

Nuclei contain electron dense material, especially next to the nuclear envelope except where there are pores (Fig. 7). One or two round electron dense bodies are usually present, which have a coarser granularity than the other electron dense material in the nucleus.

The sarcoplasm contains many clusters of glycogen in scallops fixed in April and May (Fig. 8).

DISCUSSION

The appearance of the muscle varied according to the solution which was used to dissolve the fixative. The mitochondria were comparable to those seen in other tissues when fixed using sea water, and the sarcoplasmic reticulum was more complete in those preparations. The myofibrils were not affected so much by differences in fixation. The fixatives dissolved in phosphate buffer were designed for vertebrates, whose body fluids are hypotonic to those of molluscs. The body fluids of most molluscs are almost isotonic with sea water (Prosser, 1952). The poor fixation which resulted when phosphate buffer was used was probably due to swelling of the components of the sarcoplasm in the hypotonic fixing solution.

We found that the scallop striated muscle consisted of uninucleate cells, as described by Kawaguti and Ikemoto (1958). The lack of large amounts of connective tissue between the cells, and their ability to coil up independently of each other suggests that the cells are loosely connected, which confirms Kawaguti and Ikemoto's finding (1958) that they are macerated easily. The muscle fibril is similar in its organisation to a slow arthropod muscle (Brandt et al., 1965). The fibril is made up of two types of myofilament which form A and I zones as in arthropod muscle, and the Z line has the same type of organisation. The proportion of actin to myosin filaments (8 to 1) is more similar to that of slow arthropod muscle (6 to 1) (Hagopian, 1966) than to that of fast arthropod fibres (3 to 1) (Jahromi and Atwood, 1967; Smith, 1965; Smith, 1966). The sarcomere length in scallop striated muscle, 1.9 μ, is closer to fast arthropod muscle (Jahromi and Atwood, 1967) or vertebrate muscle (Bishop and Odense, 1967). Invertebrate muscles with longer sarcomeres usually have a higher ratio of actin to myosin filaments than those with shorter sarcomeres. Hagopian (1966) suggests that the higher proportion of actin filaments gives the muscle with long sarcomeres greater tensile strength. Scallop striated muscle seems to be an exception since it has a high ratio of actin to myosin filaments and a short sarcomere length. The scallop muscle may require a high tensile strength regardless of sarcomere length because the valves provide a high resistance to the water in swimming movements.

Many mollusc muscles contain paramyosin filaments. Paramyosin filaments are characterised by an axial periodicity of about 145 Å; the diffraction picture produced by low-angle X-rays (Hanson and Lowy, 1961); and by a high proportion of tropomyosin A to actomyosin. High proportions of tropomyosin A are found in the smooth muscle which forms the slow portion of the adductor muscle in scallops and other molluscs (Elliott et al., 1957). The striated muscle of the scallop, however, contains less than 1 part tropomyosin A to 75 parts actomyosin (Ruegg, 1961); and 1 part tropomyosin B to 9 parts actomyosin, which is about 11% of the total myofibrillar protein and is comparable with the 10–12% found in rabbit skeletal muscle (Perry, 1961). The thick filaments of *Placopecten* (Fig. 3) look very similar to the rabbit myosin aggregates described by Huxley (p. 56, 1964), and show no signs of periodicity. A spiral periodicity described by Samosudova and Slavskaya (1961) was not seen. The thick filaments of scallop striated muscle are probably, therefore, myosin filaments. Since the smooth muscle of *Gryphaea angulata* (Hanson and Lowy, 1959) and the oblique muscle of *Crassostrea angulata* (Hanson and Lowy, 1961) which form the fast part of the adductor muscles in these animals contain paramyosin filaments, it would be interesting to know whether the fast adductor muscles of other monomyarian pelecypods contain paramyosin or myosin. The type of muscle seems to be associated with its function, since *Pinna attenuata* and *Atrina japonica* have a smooth translucent muscle and are sessile, the scallops have striated muscle and perform active swimming movements, and *Spondylus cruentus* with partly obliquely striated muscle is sessile, but periodically ejects water in a vigorous fashion.

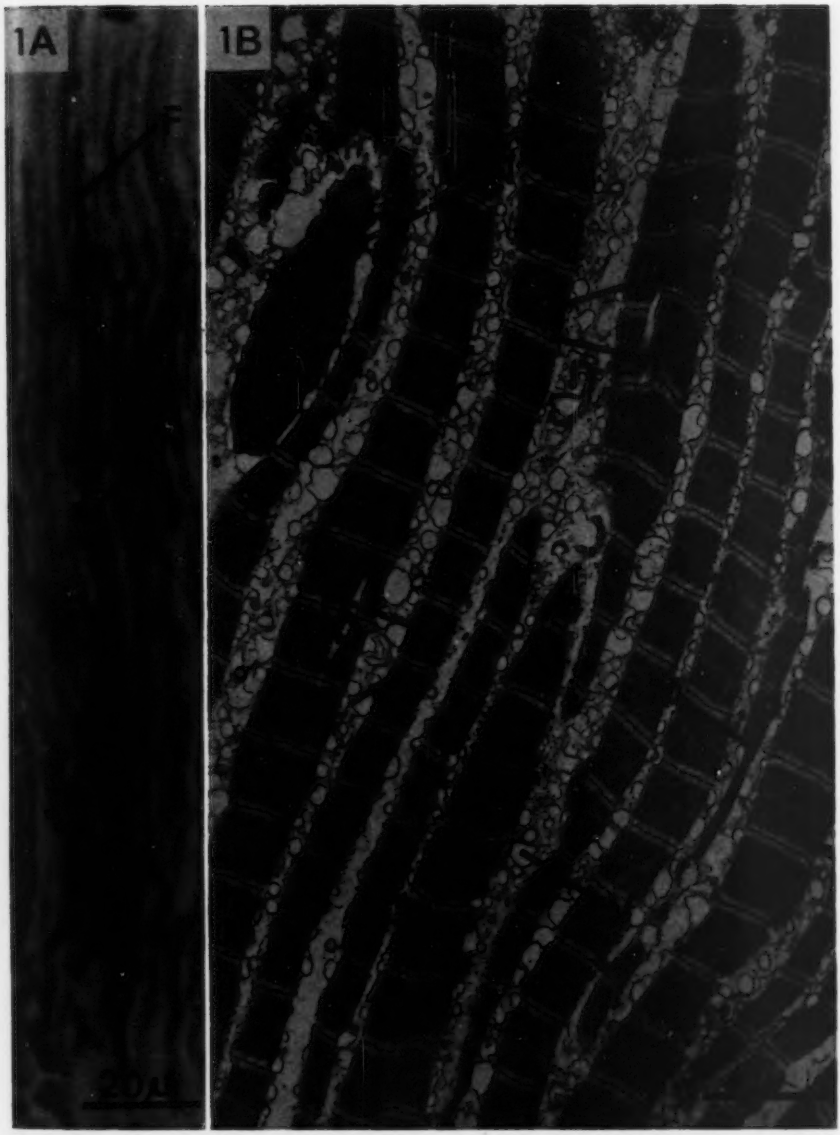

FIG. 1. A: The muscle cell is fusiform with a central nucleus (N) and striated muscle fibril (F) which runs the length of the cell. B: The striations are divisible into A and I zones. There is an H band in the centre of the A zone and a Z band in the centre of the I zone. Mitochondria (M) and a nucleus (N) are also shown, and there is a system of vesicles and tubules (V) under the cell membrane.

NOTE: Figure 1A is a light micrograph of an approximately 0.5-μ section of a specimen embedded in Araldite for electron microscopy. All other figures are electron micrographs. Figures 1, 2, 4A–C, and 8 are micrographs of material fixed using 0.05 M phosphate buffer. The other micrographs were taken of material fixed using filtered sea water.

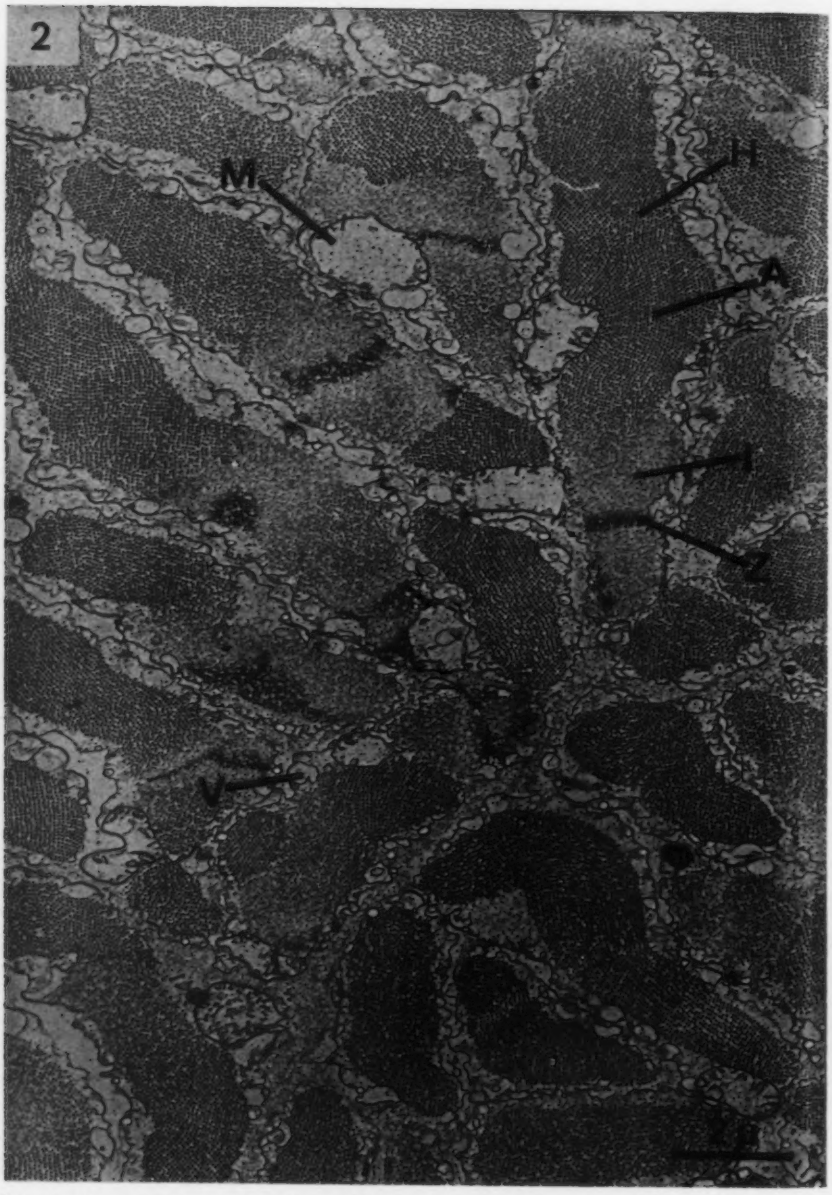

FIG. 2. Transverse section of the muscle cells shows A and I zones and H and Z bands. The mitochondria (M) are more expanded than those in Fig. 1A. Vesicles (V) are evident next to the sarcolemma.

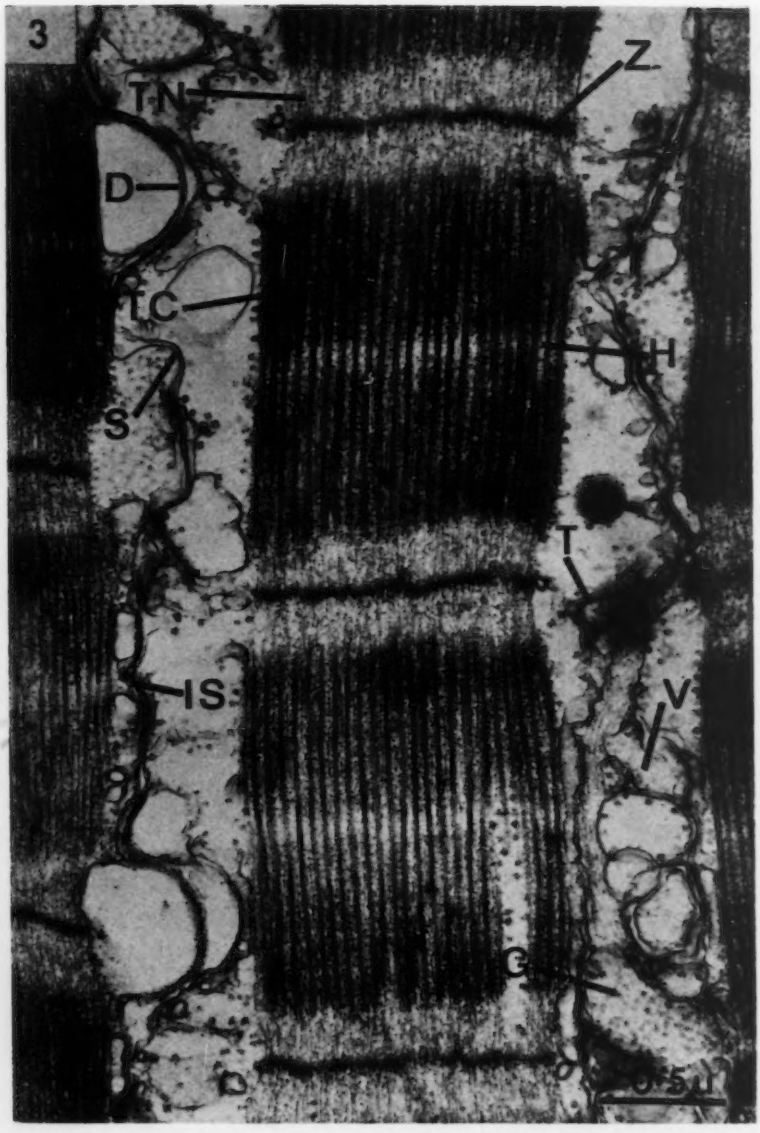

FIG. 3. At this magnification the I zone is seen to consist of thin filaments (TN). These overlap and have cross-connections with thick filaments (TC) in the A zone, except for the H band in the centre of the zone. The Z band shows no zig-zag appearance. Vesicles (V) and tubules (T) are present near the sarcolemma. The vesicles form dyads (D) with the sarcolemma (S). The intercellular space (IS) is small and contains little material. There is some glycogen (G) in the sarcoplasm.

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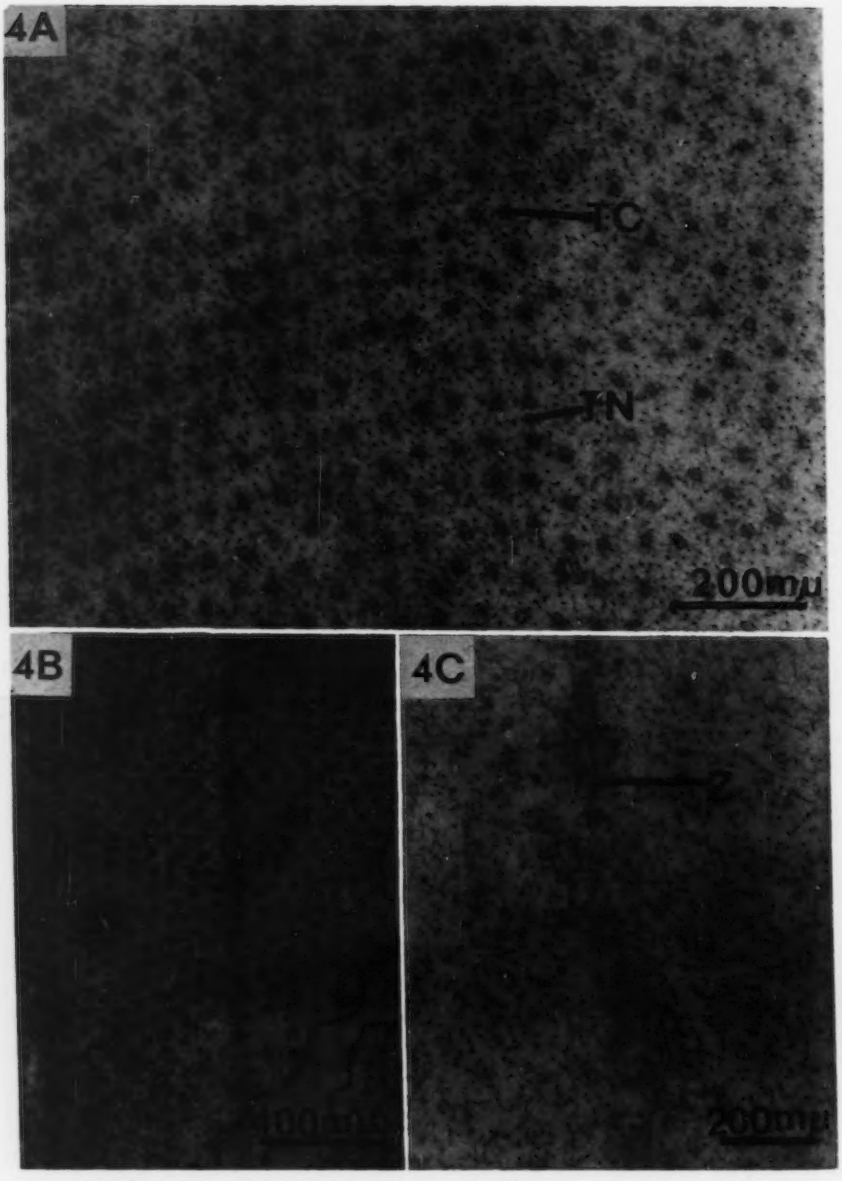

FIG. 4. A: In transverse sections of the A zone in the region of overlap of the thick and thin filaments, each thick filament (TC) is surrounded by about eight thin filaments (TN). B: An oblique section through the H zone shows that there are few thin filaments in this region. C: The Z band does not have a regular arrangement in oblique sections, but the filaments are thicker than in the rest of the I zone, and there are some signs of cross connections.

FIG. 5. The continuity of the vesicles (V) and tubules (T) next to the sarcolemma is evident in this micrograph. The vesicles sometimes form dyads (D) with the sarcolemma.

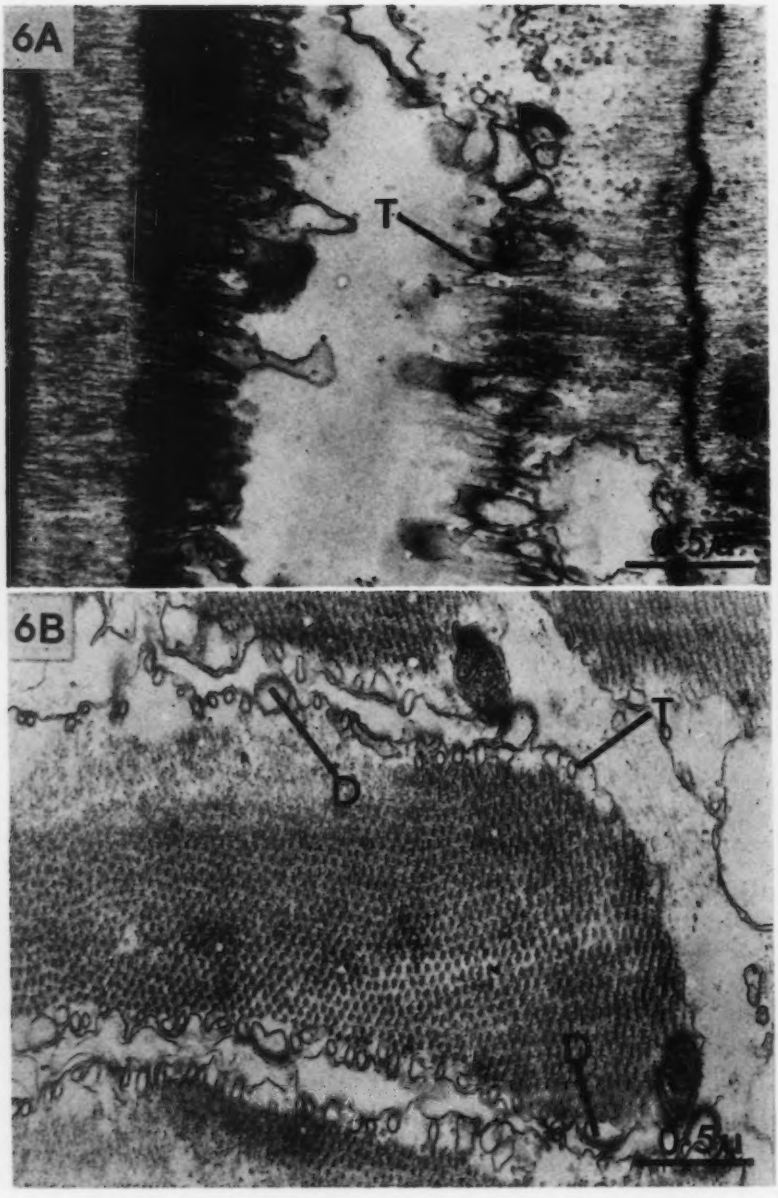

FIG. 6. A: The tubules (T) of the sarcoplasmic reticulum show a mainly longitudinal orientation. B: In transverse section the tubules (T) of the sarcoplasmic reticulum appear mainly as circular profiles. Some vesicles form dyads (D) with the sarcolemma.

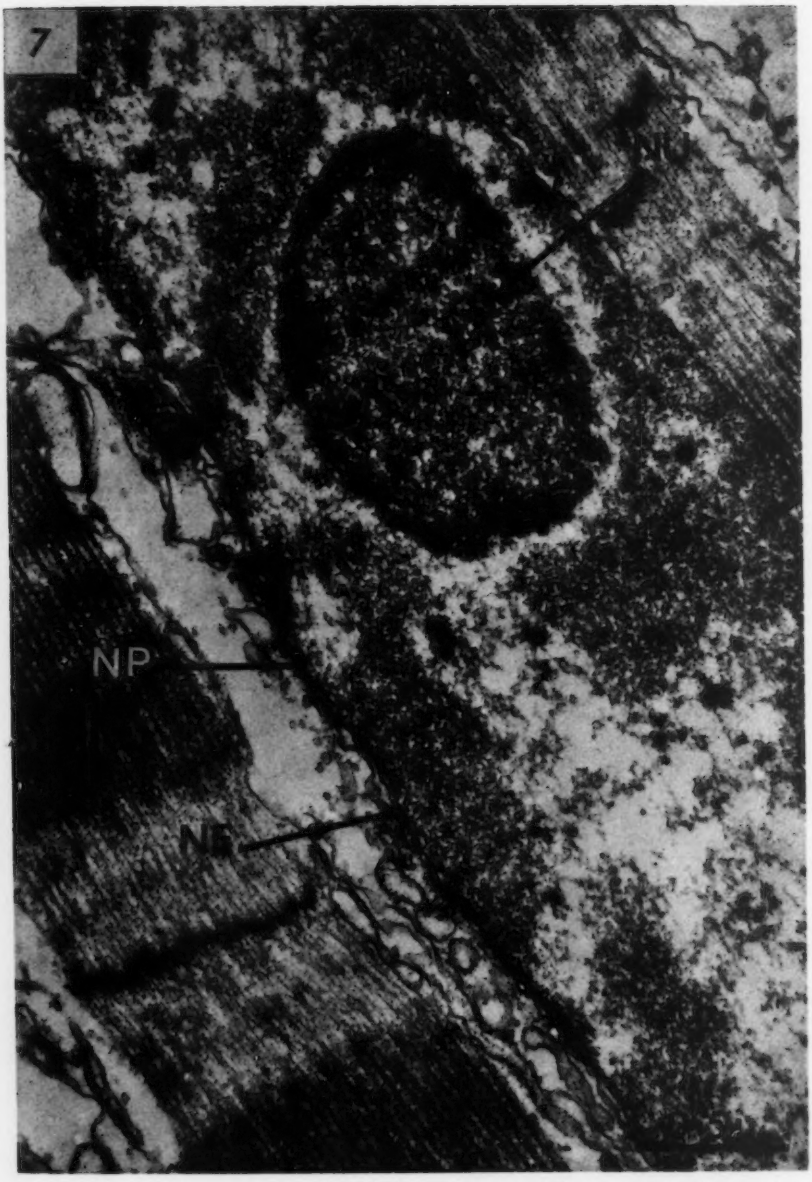

FIG. 7. There is electron dense material in the nucleus, especially next to the nuclear envelope (NE) except where there are pores (NP). The nucleolus (NU) is rounded, and shows a coarser granularity than the other electron dense material in the nucleus.

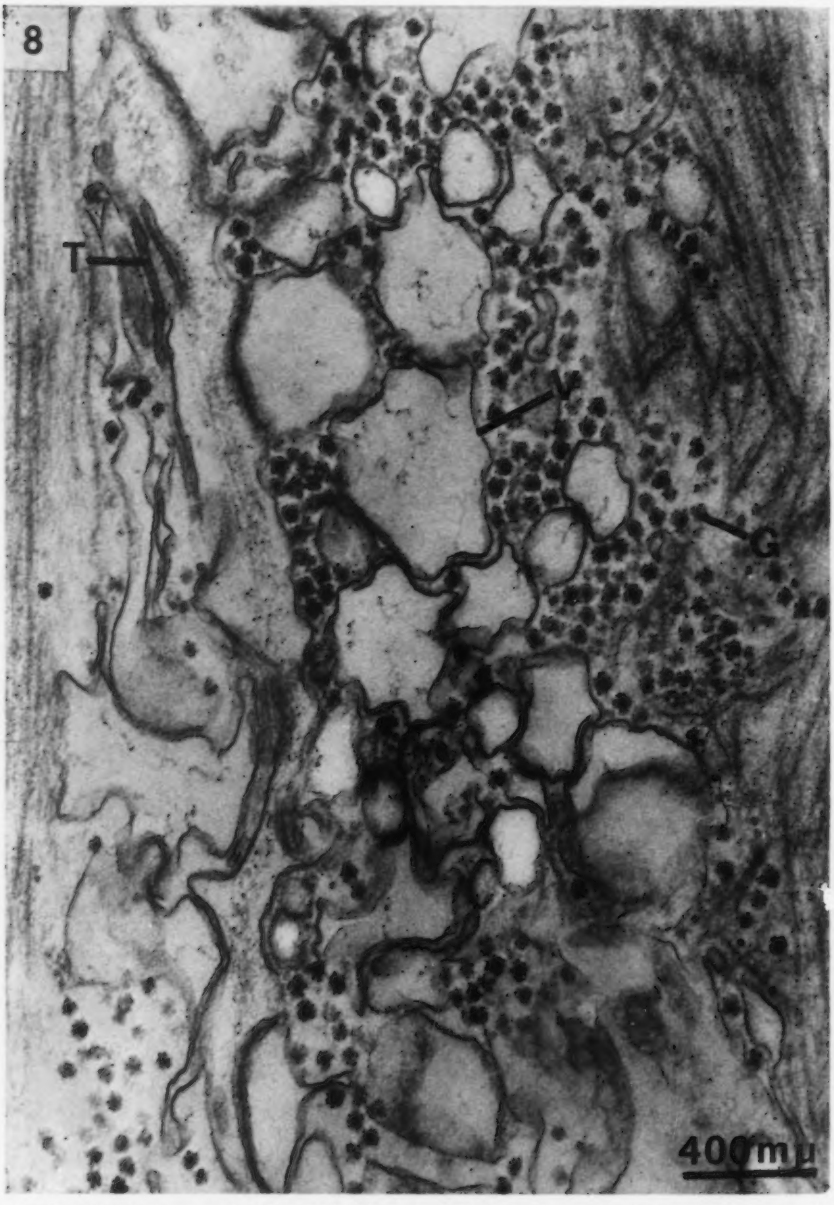

FIG. 8. Muscle from material fixed in April and May shows many clusters of glycogen (G) in the sarcoplasm. Tubules (T) and vesicles (V) of the sarcoplasmic reticulum are evident.

A possible interpretation of the morphology of the scallop sarcotubular system is shown in Fig. 9. The sarcolemmal invaginations found in arthropods and vertebrates are absent, probably because the muscle cells of these groups are syncytial, whereas those of the scallop are unicellular. The scallop sarcoplasmic reticulum is close to the sarcolemma and the myofilaments and dyads are formed between the sarcoplasmic reticulum and sarcolemma as

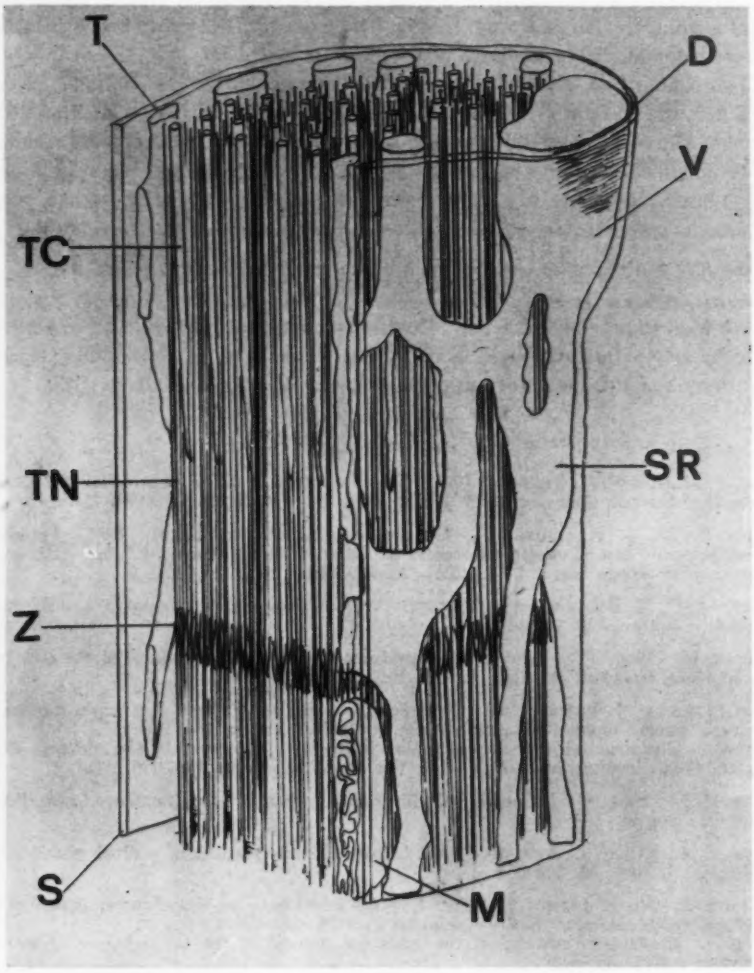

FIG. 9. This diagram gives some idea of the relationships between the cell components described in this account. The components are the thick (TC) and thin (TN) myofilaments, the Z band, the tubules (T), and vesicles (V) of the sarcoplasmic reticulum (SR), the dyads (D), sarcolemma (S), and mitochondria (M).

in arthropods. The triads of vertebrates are believed to be the sites at which depolarisations of the sarcolemma may initiate release of calcium from the sarcoplasmic reticulum. This causes contraction of the myofilaments (Philpott and Goldstein, 1967). The sequence of events could be very similar in scallop muscle, except that the membrane depolarisation would not need to be carried into the cell by invaginations since the cell is so small, and the sarcoplasmic reticulum could be directly affected at the dyads. Since vesicles which form dyads with the sarcolemma have also been described in oyster translucent adductor muscle (Hanson and Lowy, 1961) other pelecypods may possess a similar sarcoplasmic reticulum.

The amount of glycogen appears to be much lower in scallops fixed in December and January than those fixed in April and May. The first group had been kept in tanks for a longer time, so may not have received enough food to accumulate glycogen. On the other hand the scallops spawn in August to September, and the glycogen level has been found to be higher before spawning (Power et al., 1964).

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Dynamics and Exploitation of Mature Walleyes, *Stizostedion vitreum vitreum*, in the Nipigon Bay Region of Lake Superior[1,2]

By R. A. Ryder

Ontario Department of Lands and Forests
Port Arthur, Ontario

ABSTRACT

Walleye stocks in Nipigon Bay of Lake Superior were homogeneous with those in tributary inland waters but were discrete from Black Bay stocks. Returns from 2200 tagged walleyes in Lake Superior and tributary inland waters between 1955 and 1958 varied from 7.8 to 31.0% for 2 years after release. The commercial fishery in Lake Superior recovered 64.9% of the tags, the sports fishery in inland waters captured 27.6%. Fish tagged in the Nipigon River travelled a mean distance of 11.8 miles from the point of release and were recovered in 191 days (average). Total mortality rates for Nipigon Bay walleyes were 55.0% (1955–57). Mature walleyes on the spawning grounds in the Nipigon River in 1957 were estimated at 22,000, and fish in Nipigon Bay over 14 inches (total length) the same year at 41,000. All male walleyes were mature at 15 inches and females at 18 inches. Walleyes exploitation rates increased with the decline of the lake trout fishery. Wounding and scarring rates by sea lampreys increased during 1955–57 but never exceeded 1.0% on adult walleyes. Severe pollution on the west side of Nipigon Bay originated from a kraft mill. High concentrations of total solids and dense sedimentation of wood fibres created an environment unfavourable to *Hexagenia limbata* and *Pontoporeia affinis*. The recent elimination of the walleye fishery in Nipigon Bay is most likely attributable to industrial pollution rather than to overexploitation or sea lamprey predation.

INTRODUCTION

A PRECIPITOUS DECLINE in the walleye, *Stizostedion vitreum vitreum*, population of Nipigon Bay and contiguous inland waters during 1958–67 necessitated an evaluation of all available data to determine the possible causes. Data were obtained on discreteness of the stocks, migrations, distribution, survival, and population levels as well as on the sport and commercial fisheries, sea lamprey (*Petromyzon marinus*) predation, and the effects of pollution. The discontinuity of some of the data precluded the possibility of a definitive conclusion for the disappearance of the walleye population. It is hoped, however, that the presentation of all available information may help avert, or evaluate the causes for, similar walleye declines in other areas.

This study was designed originally to test the discreteness of the walleye stocks of Nipigon Bay and tributary inland waters. Because of a conflict of interests between commercial fishermen and anglers, it was desirable to determine whether two discrete populations existed: one inhabiting Nipigon Bay and exploited solely by the commercial fishery, and the other restricted

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to inland waters such as Polly Lake and the Nipigon and Jackfish rivers and harvested only by anglers. Another possibility was the intermingling of heterogeneous stocks after spawning although mature fish homed to discrete spawning grounds (e.g. Crowe et al., 1963).

TAGGING EXPERIMENT

METHODS

A total of 2200 adult walleyes were tagged in the Nipigon Bay and Black Bay watersheds of Lake Superior in 1955–58 (Table I). All fish (10.1–23.7

TABLE I. Numbers of walleyes tagged and numbers and percentages recaptured in the Nipigon Bay region of Lake Superior and contiguous inland waters from 1955–58.[a]

Tagging site	Year	Method of capture[b]	No. tagged	Tag returns	
				No.	Percentage
Nipigon River	1955	D	90	7	7.8
" "	1956	D, S	1000	206	20.6
" "	1957	D	504	160	31.7
Totals	1955–57	D, S	1594	373	23.4
Polly Lake	1955	A	30	6	20.0
Polly Lake	1956	A	25	3	12.0
Totals	1955–56	A	55	9	16.4
Lake Helen	1955	G	15	0	0.0
Jackfish River	1955	A	2	0	0.0
Black Bay	1958	G	534	15	2.8
Grand totals	1955–58	D, S, A, G	2200	397	18.0

[a]This study does not include one walleye tagged in Black Bay on May 15, 1958, and recovered at the mouth of Black Bay in October 1964. This fish, at large for over 6 years, was the only one recovered more than 3 years after tagging.
[b]D = dipnet, S = seine, A = angling, and G = gillnet.

inches total length (TL)) were marked with streamer-type tags in the manner outlined by Joeris (1953). Walleyes for tagging were captured principally by dipnet, although some were obtained from seines and gillnets, or by angling. They were anesthetized with M.S. 222 (tricaine methanesulfonate), urethane, or ether; measured (TL); sexed when possible; tagged and released at five sites. Fish marked in the Nipigon River were captured at night during the spawning run in late April and early May. Walleyes marked elsewhere were tagged in the daytime. All fish were taken randomly within the limitations of the capturing gear. Dipnetters did not select for particular fish although those in shallow water and closest to the tagging site were invariably captured first. Tagged fish recaptured on the spawning grounds during the 2 weeks of tagging each spring were released and not tallied as recaptures unless originally tagged in a previous year.

Reporting of tag recaptures was encouraged by several methods. Local newspapers on several occasions publicized the marking project and encouraged the return of tags by reporting on individual recaptures and the progress of the project. Fish and game clubs cooperated by collecting tags. Tourist outfitters and camp operators were provided with mimeographed instructions indicating where the tags should be forwarded, and the data required from each capture. These instructions were also posted at various points of access to the watershed. Minimal data requested with each tag return included tag number and date, and location and method of capture. Length, weight, sex, condition of gonads, and scale samples were also obtained when recaptured fish were provided. An additional check on recoveries in the sport fishery recorded all marked and unmarked walleyes caught by angling in Polly Lake during 1957. Commercial fishermen on Nipigon Bay and Black Bay, and fish buyers and packing houses were canvassed periodically by conservation officers for tags and pertinent data. Rewards were not offered for the return of tags, although each tag was acknowledged by letter, which provided data on the locality and date of release, minimum distance travelled, and total length at time of tagging.

Recovery

Of the 2200 adult walleyes tagged, 397 were recaptured and reported (Table I). A total of 373 tags were returned from the 1594 walleyes released in the lower Nipigon River in 1955–57 and 24 were from the 589 fish tagged at Polly Lake in 1955 and 1956, and Black Bay in 1958. None of the 17 fish tagged in Lake Helen and the Jackfish River in 1955 were recovered.

The tagged fish were recaptured principally by commercial fishermen and anglers (Table II). A few were recovered from an electrical sea lamprey weir or biological sampling and one was found dead.

DISCRETENESS OF POPULATIONS

A knowledge of the number of spawning runs of walleyes in Nipigon and Black bays and tributary waters is requisite to any discussion on discreteness of the stocks. Only two large spawning aggregations were located in these watersheds: one in the lower Nipigon River, and the second, a shoal-spawning stock, at the north end of Black Bay. Another spawning run in the Black Sturgeon River was insignificant in numbers compared with the two large runs. Commercial fishermen and local residents knew of no other spawning runs in the watershed. An attempt to locate other spawning grounds in Black and Nipigon bays, Polly Lake, and the Nipigon River was unsuccessful.

The heterogeneity of the stocks inhabiting Nipigon and Black Bays was tested by marking walleyes on the two spawning grounds and observing the locations in which the tagged fish were returned. On the basis of these alone, the stocks in the two areas were discrete, with only occasional interchanges of small numbers of fish. Of 373 tag returns from 1594 walleyes released

TABLE II. Numbers of tagged walleyes recovered by various methods from those released in the lower Nipigon River from 1955 to 1957, and percentages of the total recovered.

Recovery method	Returns	
	No.	Percentage
Commercial fishery, total	242	64.9
Gillnets	165	44.2
Poundnets	77	20.6
Sport fishery	103	27.6
Biological sampling, total	20	5.4
Dipnets	15	4.0
Gillnets	5	1.3
Other, total	8	2.1
Sea lamprey weir	7	1.9
Dead on beach	1	0.3
Grand total	373	–

on the spawning ground of the lower Nipigon River, only 4 were recaptured in Black Bay (Fig. 1). All 15 returns from 534 walleyes tagged in Black Bay were made in Black Bay (Fig. 2).

Walleye stocks in Nipigon Bay and Polly Lake were homogeneous. Tagging in these two locations showed a free interchange of walleyes. Of the 373 tag returns from walleyes released in the lower Nipigon River, 83 were recaptured in Polly Lake (Fig. 1). Two of the nine recaptures from 55 walleyes tagged in Polly Lake were made in Nipigon Bay (Fig. 2).

MIGRATION

Walleyes tagged in the lower Nipigon River in 1955–57 showed similar seasonal movements from year to year. Walleyes on the Nipigon River spawning grounds in May during these 3 years reappeared on the spawning grounds 1 year later, almost to the day (Fig. 3). Concentrations of walleyes at the northwest corner of Nipigon Bay, the Clay Banks, and Polly Lake also occurred about 1 year apart suggesting a dispersal which was repeated annually.

Walleyes were first encountered in the Nipigon River in late April before spawning, which usually took place in early May near the townsite of Nipigon (Fig. 1). Shortly after spawning they left the vicinity of the spawning grounds and dispersed: (1) upstream through Lake Helen and into Polly Lake, which some fish reached in late May, the majority by June; (2) downstream to Nipigon Bay. In the downstream movement, the first fish arrived at Five Mile Point in Nipigon Bay by late May and followed the west shore in a southerly direction. Some of these fish even reached Black Bay as shown by

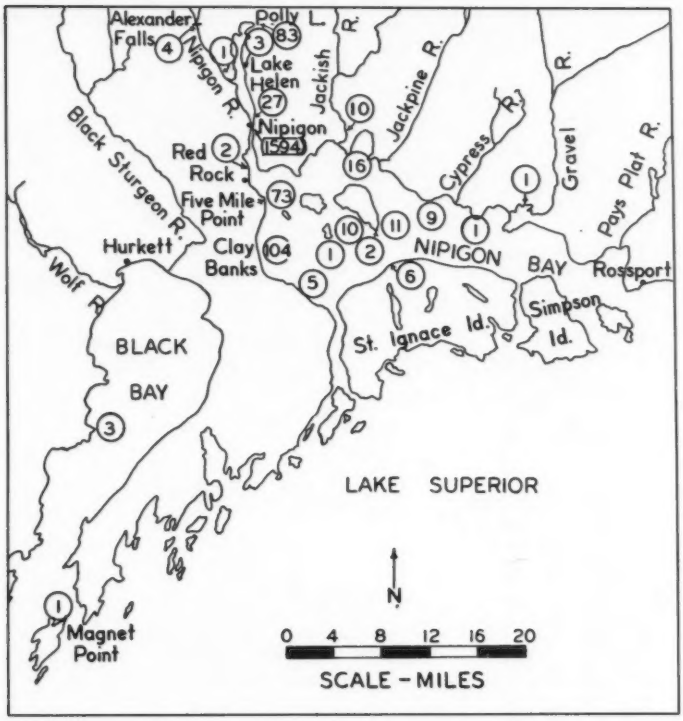

FIG. 1. Recapture locations of 373 mature walleyes tagged in the lower Nipigon River during the spawning runs of 1955–57. The box indicates the tagging locality and numbers of walleyes tagged (1594).

the four tag returns from that location. The deep, cold water between Nipigon Bay and Black Bay evidently constitutes an effective ecological barrier to large interchanges of walleyes between these two areas. By the end of June walleyes in the downstream movement were widely distributed in the shallow west end of Nipigon Bay and some of its tributaries on the north shore.

RATE OF TRAVEL AND TIME LAPSE

The 373 recaptured walleyes from the Nipigon River tagging were taken a mean distance of 11.8 miles from the release point (Table III). These ranged from walleyes recaptured at the point of release in subsequent years and recorded as 0 miles travelled, to those tagged in the lower Nipigon River and recovered in Black Bay. The exact points of recovery of three recaptures from Black Bay were not known, and they were considered to have been captured half way between the mouth and the lower end of Black Bay, or 76 miles from the point of release (Fig. 1).

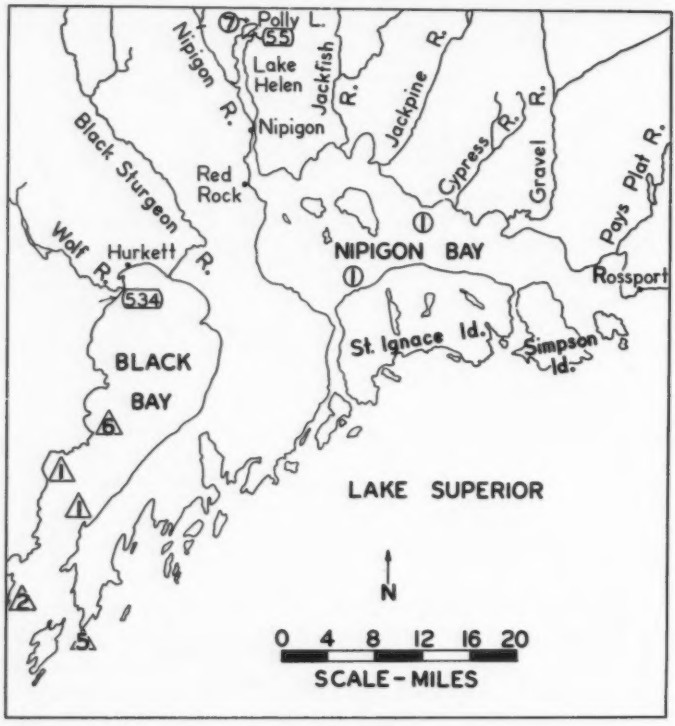

FIG. 2. Recapture locations for 9 walleyes tagged in Polly Lake (circles) and 15 tagged in Black Bay (triangles). Numbers in boxes indicate the numbers of fish tagged at the two localities.

Without respect to the location of the tagging site, the largest single category of walleyes was recaptured 6–10 miles from the point of release (Fig. 4). These were fish tagged in the lower Nipigon River and recovered on the west side of Nipigon Bay at Five Mile Point, or in Polly Lake (Fig. 1). The next largest group (11–15 miles from the tagging site) were primarily fish recovered near the Clay Banks in Nipigon Bay, after being released in the lower Nipigon River. Walleyes recovered 16–30 miles from the point of tagging were captured mainly in central and eastern Nipigon Bay. Of the 38 fish in the 0–5-mile category, 35 were captured less than 1 mile from the release point. These were mainly fish which were taken in the lower Nipigon River during spawning runs in subsequent years, or by anglers from fish which were both tagged and recaptured in Polly Lake. The four fish which moved the greatest distance (61–80 miles) were tagged in the Nipigon River and recaptured in Black Bay.

Most walleyes undoubtedly moved further than the minimum recorded distances would imply. Fish recaptured at the tagging site on the spawning

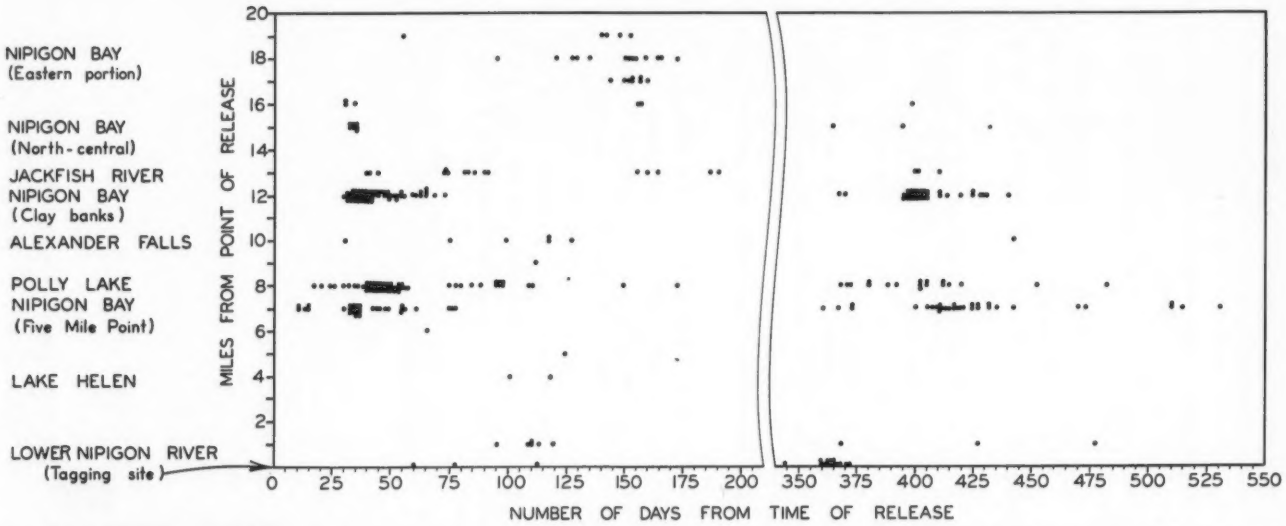

FIG. 3. Distances moved from tagging site and numbers of days from release to recovery for 358 walleyes which were tagged in the lower Nipigon River during the spawning runs of 1955–57. Of the total of 373 recaptures, only tags recovered during the first 2 years after release are included. Each recapture is represented by a dot. Six tags recovered over 20 miles from the point of release and not shown on the graph include four from Black Bay, one from eastern Nipigon Bay, and one from the Gravel River (Fig. 1).

TABLE III. Distances moved and times between release and recapture of 397 tagged walleyes.

Year	Tagging site	No. of fish recovered	Mean distance from release point (*miles*)	Mean time lapse (*days*)
1955	Nipigon River	7	6.8	142.7
1956	" "	206	11.5	255.6
1957	" "	160	12.5	110.3
Subtotals	" "	373	11.8	191.2
1955	Polly Lake	6	4.3	12.3
1956	" "	3	9.0	53.7
Subtotals	" "	9	5.8	26.1
1958	Black Bay	15	21.2	254.1
Grand total	All waters	397	12.0	189.8

grounds in the lower Nipigon River in subsequent years had probably moved to Polly Lake or Nipigon Bay in the interim. The distance moved, however, was recorded as 0 miles rather than the more probable minimum distance of 40 miles.

The 373 tag recoveries of fish tagged in the Nipigon River were made at an average of 191.2 days, or slightly more than half a year, after release (Table III). These fish ranged from two which were captured in poundnets at Five Mile Point 11 days after their release, to one which was recovered at the same location 879 days (or about $2\frac{1}{2}$ years) after release. There was a tendency for fish tagged at one location on the same night to be recovered together on about the same date, even after considerable lapse of time. The walleyes were particularly vulnerable to capture at certain locations, and if only the recaptures

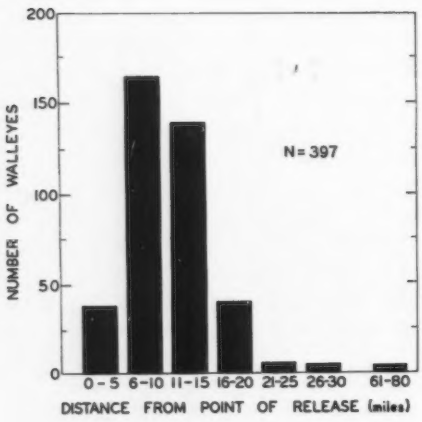

FIG. 4. Numbers of tagged walleyes, out of the 397 recovered, that were taken various distances from the point of release.

made within 2 years of tagging are plotted, a generalized picture of annual movement becomes evident (Fig. 3). The concentrations of points in Fig. 3 are also indicative of the principal locations of the commercial and sports fisheries. Two of the major concentrations of recaptures shown in Fig. 3 were from the commercial fishery in western Nipigon Bay. The third area with large numbers of recaptures was Polly Lake, where most fish were taken by anglers.

The first-year relationship between distance moved and number of days to recovery is shown in Table IV, where only the major recovery sites are considered. The rate of travel from the lower Nipigon River downstream to

TABLE IV. Distances moved from tagging site, numbers of days from release to recovery, and minimum rates of travel for 202 tagged walleyes recaptured within 1 year after release in the lower Nipigon River. Only the six major recapture areas are considered.

Recapture area	Current direction from tagging site	No. of 1st year tag returns	Distance from tagging site (*miles*)	Mean no. of days to recapture	Rate of travel (*miles/day*)
Five Mile Point	Downstream	25	7	48.0	0.15
Polly Lake	Upstream	59	8	58.0	0.14
Clay Banks	Downstream	67	12	44.1	0.27
Jackfish River	Downstream and upstream	9	13	76.8	0.17
Eastern Nipigon Bay	Downstream	27	18	144.2	0.12
Lower Nipigon River	Downstream and upstream	15	36[a]	364.2	0.10
All sites	Downstream and upstream	202	12.8	87.2	0.18

[a]Tagged fish recovered in the lower Nipigon River 1 year after tagging were considered to have followed the minimal cyclic movement to eastern Nipigon Bay (18 miles) and returned to the point of tagging.

the Clay Banks in Nipigon Bay was estimated at 0.27 miles per day; upstream to Polly Lake, 0.14 miles per day. Table IV shows a trend toward diminishing mean rates of travel as days to recapture increased. This was doubtless caused by the cumulative effect of route deviations with increase in time from release. The maximum rate of travel recorded was for two walleyes that moved 7 miles downstream in 11 days, or 0.64 miles per day (Fig. 3).

IMPORTANCE OF THE FISHERIES

SPORT FISHERY

Approximately 80% of the tags returned from the sport fishery were taken in Polly Lake; almost all the remaining 20% were taken in the Jackfish

and Nipigon Rivers, relatively few being from Lake Helen and the Gravel River. Angling specifically for walleyes in Nipigon Bay does not normally occur, and only rarely is one taken incidental to the lake trout sport fishery.

A complete creel census on Polly Lake in 1957, then, was expected to account for a large proportion of the sport fishery harvest in that year. At the time of the creel census only one major access point was available on Polly Lake, and the posting of a full-time census taker there provided complete records. Angling on Polly Lake takes place from the opening of the season (May 15 in 1957) to the end of August. Fishing is extremely poor by early September and angling occurs only rarely in the fall and winter.

A total of 1517 walleyes were captured in Polly Lake by 1460 anglers, at a rate of 0.33 fish per hour (Table V). If this is considered as 80% of the catch

TABLE V. Summary of complete creel census for Polly Lake, from May 15 to August 31, 1957.

Month	No. of anglers	No. of man-hours of fishing	Walleyes caught by anglers				
			No.	No. per hour of angling	Mean total length (*inches*)	Weight (*lb*)	
						Mean	Total
May	77	224	43	0.19	17.2	1.75	73
June	477	1502	441	0.29	16.8	1.56	658
July	402	1261	354	0.28	17.3	1.75	624
Aug.	504	1549	679	0.44	17.6	1.88	1263
Total	1460	4536	1517	0.33	17.3	1.75	2618

for the whole watershed, based on the ratio of tag returns, then the calculated total angling catch was 1896 walleyes.

More walleyes were caught in Polly Lake in August than in other months (Table V). Both effort (1549 man-hr) and catch per unit of effort (0.44 per hour) were highest in August.

Mean total lengths and weights of walleyes entering the sport fishery in Polly Lake varied slightly from May to August (16.8–17.6 inches; 1.56–1.88 lb).

COMMERCIAL FISHERY

Long-term trends in the commercial fishery of Nipigon Bay were determined from data on production, fishing intensity, and abundance. Production was expressed as landed catch, fishing intensity as an index number proportional to effort (the number of yards of gillnets, 4½-inch stretched measure, lifted in a 24-hr period, or the number of poundnet lifts for a similar period), and abundance as an index number proportional to the mean catch

per unit of effort. Production records were available for 21 years (1945–65), fishing intensity and abundance data being available for only the last 17 years.

The walleye fishery in Nipigon Bay up until 1957 constituted only a small portion of the total catch of the three major species, which included lake trout and lake whitefish, *Coregonus clupeaformis* (Fig. 5A). In 1957 and 1958,

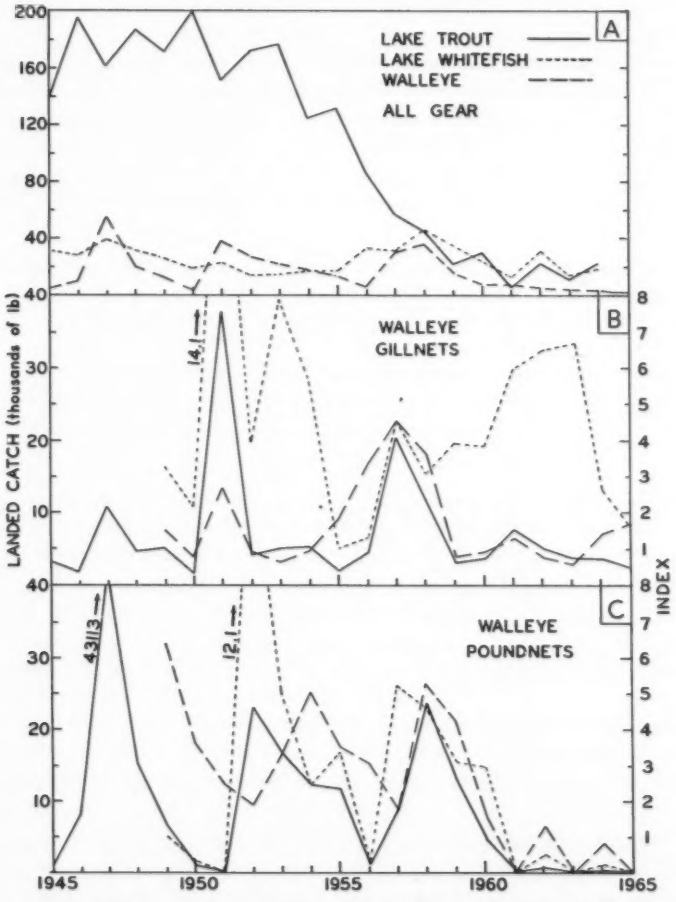

FIG. 5. Fish production data for Nipigon Bay, 1945–65. (A) Landed catches of lake trout, lake whitefish, and walleyes in gill- and poundnets. (B) Walleye production (solid line), fishing intensity index (long dashes), and abundance index (short dashes) for the gillnet fishery. (C) Walleye production (solid line), fishing intensity index (long dashes), and abundance index (short dashes) for the poundnet fishery. As no walleyes were captured in 1961, 1963, and 1965, the fishing intensity and abundance indices are recorded as zero.

the walleye production, although not greater than in 1947 and 1951, nevertheless contributed a substantial proportion of the total catch, due in part to the rapid decline of the lake trout fishery. Fishing intensity for walleyes, however, reached a high level for both gill- and poundnets during these same 2 years (Fig. 5B and 5C), marking a shift in the direction of fishing intensity from lake trout to walleyes. This shift in fishing intensity was a practical one in Nipigon Bay, where the adult walleyes moved over the same route each year and were concentrated in late May and June at Five Mile Point and the Clay Banks after spawning.

These concentrations of adult walleyes made them particularly vulnerable to the gill- and poundnets for a short period of time each spring. During 1957 and 1958, when the walleye production was at a relatively high level, 80% (23,186 lb) and 84% (29,684 lb) respectively, of the total walleye catch was taken during the period starting late in May and continuing through June (Table VI). Poundnets were traditionally set between Five Mile Point and

TABLE VI. Distribution of walleye catch (in pounds) by month for both gill- and poundnets in Nipigon Bay, 1954–65.

Year	Jan.–Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1954	169	3,225	7,453	5,821	16	91	185	472	60
1955	2	449	2,626	588	5,195	3,618	413	485	–
1956	–	87	18	249	457	1,367	2,404	885	–
1957	–	90	23,096	5,655	–	85	16	6	–
1958	159	5,992	23,692	3,695	–	1,095	541	62	–
1959	–	7,463	8,137	214	–	–	–	–	–
1960	–	1,467	6,461	318	–	–	–	–	–
1961	–	2,060	5,134	253	–	–	–	–	30
1962	1,830	1,584	2,127	–	–	–	18	–	–
1963	–	654	2,931	–	–	–	5	141	–
1964	–	460	2,578	313	–	–	447	73	–
1965	–	198	2,191	–	–	59	–	11	–
Total catch	2,160	23,729	86,444	17,106	5,668	6,315	4,029	2,135	90
Mean catch	540	1,977	7,204	1,901	1,889	1,052	504	267	45

the Clay Banks (Fig. 1) and cropped the spawning stock as they descended southward in Nipigon Bay along the west shore. As there has been relatively little movement of poundnets over the years, abundance estimates obtained from them (Fig. 5C) probably reflect the true population levels of walleyes. The extremely high catch per unit of effort for gillnets in 1959–63 (Fig. 5B) likely illustrates increased efficiency in capture rather than actual high abundance of walleyes. The commercial fishermen evidently fished walleyes more effectively as they became attuned to the fish's seasonal movements. The

sharp drop in catch per unit of effort for gillnets in 1964 and 1965 indicates a further marked decline in the few remaining walleye stocks. The increase in fishing intensity for gillnets in 1964–65 (Fig. 5B) may have resulted from a re-direction of effort in those years to a slightly improving lake trout fishery.

The production of walleyes in Black Bay over the same period (1945–65) increased gradually except for one sharp increase in 1959 (Fig. 6). The production for Black Bay was closely associated with fishing intensity and bore

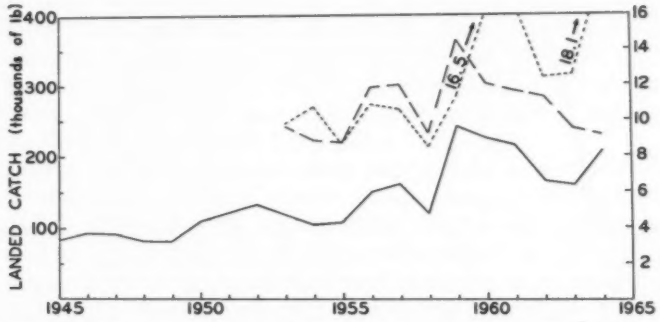

FIG. 6. Walleye production (solid line) for the pound- and gillnet fisheries of Black Bay. The fishing intensity index (long dashes) and abundance index (short dashes) are recorded for the gillnets only, which constituted the major part of the fishery.

no symptoms of an impending decline. Production was seven or eight times greater on the average during 1945–65 than that of Nipigon Bay and reflected the quite different environmental situations in the two bays. Black Bay is shallow with a mean depth of about 20 ft, whereas Nipigon Bay is more typically lake trout habitat with a mean depth of about 90 ft. Most of the walleye catch in Nipigon Bay was taken from the shallow west end; the remainder of the walleye catch from the deep east end was usually taken from shoals, or close to shore on the mainland or islands.

As the walleyes in Black Bay are shoal spawners, the brood stock is scattered over a considerable piece of shoreline. Consequently they are not as vulnerable to commercial exploitation as walleyes were in Nipigon Bay in their concentrations after spawning.

The restricted habitat, concentrated spawning aggregations, and seasonal migrations of the Nipigon Bay walleyes increase their vulnerability to commercial exploitation, particularly to mobile gear.

EXPLOITATION, SURVIVAL, AND MORTALITY RATES

EFFECT OF VULNERABILITY ON TAG RECAPTURES

A vulnerability factor was computed for all tagged walleyes over 14 inches TL to determine the relative selectivity of the commercial and sport

fisheries for tagged walleyes as compared with the unmarked population. Length-frequency polygons (Fig. 7A) were plotted for samples from the commercial catch (gillnet and poundnet data pooled) and all tagged fish for the years 1955–57. The shaded area represents 18.4% of the tagged fish, which

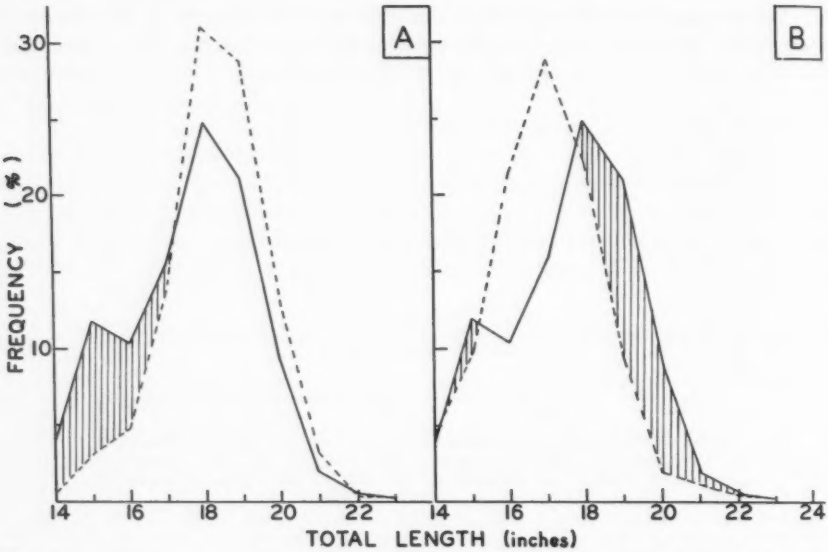

FIG. 7. Length-frequency distribution of walleyes, 14 inches TL and greater, tagged in the lower Nipigon River in the spring of 1957 (solid line). The broken line is the distribution of walleyes entering the commercial catch (A) or the angling catch (B) during the same year. The hatched areas represent the percentages of which were not vulnerable to (A) the commercial fishery (18.4%) and to (B) angling (21.9%).

were not vulnerable to the commercial fishery and were considered to be noneffective tags, following the method of Ketchen (1953). Similar polygons (Fig. 7B) plotted for all tagged and untagged fish entering the sport fishery resulted in an estimate of 21.9% noneffective tags. The total percentages of tagged fish, therefore, that could be expected to be vulnerable to the commercial and sports fisheries, respectively, are 81.6 and 78.1%. In the population and exploitation estimates a rounded vulnerability factor of 0.8 was used to correct estimates for gear selectivity.

One criterion is not satisfied in the estimates of Ketchen's vulnerability factor in this study. Tagged fish were all mature; untagged fish were both mature and immature. The vulnerability correction then, although in the right direction, is probably inadequate. Consequently, calculated exploitation rates are too low, and population estimates too high. The extent of this error cannot be estimated from available data. Trends in exploitation rates will, however, be of the same order of magnitude.

Effects of Tagging Mortality and Tag Loss

Various measures were used to attempt to hold tagging mortality at a minimum. Only small lots of walleyes were marked at one time, and all were anesthetized to reduce the probability of injury caused by rough handling of vigorous fish. Walleyes tagged in the lower Nipigon River were released in shallow water protected by a groin from which injured fish could be easily recovered with a dipnet. To reach the main river channel the tagged fish had to swim against a back eddy around the end of the groin. Scale samples were not taken from tagged fish, nor were they finclipped, thereby reducing the possibility of infection. Mutilated walleyes, or those in obviously weak condition, were not marked. Lymphocystis-infected fish, however, which made up 24.8% of the spawning population in the Nipigon River in 1956 and 29.2% in 1957, were tagged and released. The natural mortality of infected fish was not higher than for noninfected fish (Ryder, 1961).

The degree of tag loss in this study can be only crudely estimated. The only basis on which to ascertain tag loss was the wound or scar left at the point of tag attachment. Generally, rather severe erosion was noticed on tagged fish which had been at large for 2–3 months or longer. In some fish that had been at large for more than 1 year, the tag had moved upwards and backwards along the interneural spine, and continued up the rays of the soft dorsal fin, where it was held insecurely by the fin membrane. The erosion in the flesh on both sides of the body had healed, leaving a readily observable scar. Although several thousand walleyes were observed in the commercial and sport fisheries, and during biological sampling for 2 years after the 1955 and 1956 tagging in the Nipigon River, only two fish were observed with obvious tagging scars and without tags. On this basis tag loss was considered negligible for at least 1 year after tagging. For population estimates, only returns 1 year after tagging were considered, and no tag-loss correction was applied.

Calculation of Rates

Tag recoveries for 2 consecutive years after release in 1955 and 1957, and for 3 years after release in 1956, were used to compute exploitation rates (Table VII). For fish tagged in 1956, almost as many tags were recovered the 2nd year (45.6% of the total number recovered) as the 1st (50.0%); for fish tagged in 1957, 83.8% of the tags were recovered in the 1st year after release and only 16.2% in the 2nd year. Combined returns for all years showed about two-thirds of the returns made in the 1st year after release, one-third in the 2nd year, and only 2.4% in the 3rd year. Differences in rates of return from year to year depended on several variables. As tagging mortality and tag loss have already been considered, other likely influences on tag return were: location and degree of fishing intensity; annual variations in the movements of walleyes; irregularity in reporting tags; a combination of all these possibilities.

Catch curves as defined by Ricker (1958) were plotted for mature walleyes captured by dipnet and used to compute survival rates. These included only

TABLE VII. Numbers of tagged walleyes recovered in different years after release in the lower Nipigon River in 1955–57, and (in parentheses) percentages of total recoveries.

Year of tagging	No. tagged	Total no. of recoveries	No. recovered in		
			1st year	2nd year	3rd year
1955	90	7	5(71.4)	2(28.6)	Nil
1956	1000	206	103(50.0)	94(45.6)	9(4.4)
1957	504	160	134(83.8)	26(16.2)	Nil
1955–57	1594	373	242(64.9)	122(32.7)	9(2.4)

fish taken during the spring spawning runs in the lower Nipigon River in 1956–58, and were plotted as percentage age-frequency distributions for 1955–57 (Fig. 8). Scale samples were not taken from tagged fish; instead intermittent samples of walleyes were taken each night for the purpose of collecting scale samples and determining sex. As it was not apparent that the size of the fish influenced their catchability by dipnet, the catch-curves are representative of the actual age frequencies of walleyes on the spawning grounds within the limitations of the sample sizes. The ascending right limbs of the catch-curves presumably indicate incomplete maturity of both sexes until age VIII, and do not reflect gear selectivity. This supposition is supported in part by the absence of immature fish on the spawning grounds. The solid lines of Fig. 8 constituting the descending limbs of the catch-curves were plotted from the data used to compute survival rates. The datum represented by the point at age XI on the 1955 curve was not included in survival estimates as it was established on the basis of only one fish. As survival estimates from catch-curves refer to survival several years earlier, these may be properly combined with exploitation rates for 1955–57 only if constant survival is assumed. Trends in vital statistics of the Nipigon Bay walleye population as shown are categorically valid, although the absolute values shown may be in error.

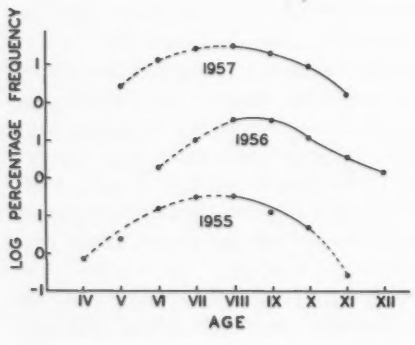

FIG. 8. Catch-curves based on scale samples from walleyes taken during the spawning runs of 1956, 1957, and 1958 in the lower Nipigon River.

Exploitation (u) and survival rates (s) were derived from the data in Tables VIII and IX. Actual survival was computed as mean weighted survival, which weights successive ages with respect to their abundance (Jackson, 1939). Computed survival rates (Table X) for the 3 years were of the same order of magnitude. A mean weighted survival rate of 0.45 was computed by combining age frequencies for all 3 years, thereby minimizing the effect of varying year-class strength. Total mortality (a) was obtained from $a = 1-s = 0.55$.

TABLE VIII. Estimates of exploitation rates, in 1st year after tagging, for walleyes over 14 inches TL.

Item	Year of tagging		
	1955	1956	1957
No. of tagged fish >14 inches at start of season	85	979	493
Corrected for vulnerability (×0.8)	68.8	783.2	394.4
No. of tagged fish recaptured in 1st year	5	103	134
Rate of exploitation (u)	0.07	0.13	0.34

TABLE IX. Data for frequency of occurrence of walleyes of different ages in 1955–57 (combined) used in survival computations.

Age:	IV	V	VI	VII	VIII	IX	X	XI	XII
No. of fish	3	15	88	176	207	111	45	6	1
Percentage of total	0.5	2.4	13.5	27.0	31.8	17.1	7.0	1.0	0.2

First year exploitation rates (u) were estimated by using Ricker's (1958) method, the fish being marked prior to the fishing season in successive years. Rate of exploitation then is equal to the number of 1st year recaptures divided by the total number of fish tagged at the beginning of the fishing season. Only tagged fish greater than 14 inches TL were included in the computations of exploitation rates, which were then multiplied by 0.8 (Table VIII) to correct for vulnerability. The exploitation rates show an increase of 85% from 1955 to 1956 and 162% from 1956 to 1957. Exploitation rates in this fishery are probably related to the gradual change from a lake trout fishery in the early 1950's to a walleye fishery by the latter part of the decade. Catches per unit of effort for gillnets during 1955–57 (Table XI) fluctuated

TABLE X. Mortality rates as computed from catch curves and tagging data for mature walleyes in the Nipigon River.

Item	Year		
	1955	1956	1957
Survival rate (s) — from catch curves	0.43	0.60	0.53
Weighted rate (s), all years combined	0.45	0.45	0.45
Annual expectations of death			
1. Total (a)	0.55	0.55	0.55
2. Fishing (u)	0.07	0.13	0.34
3. Natural causes (v)	0.48	0.42	0.21
Instantaneous mortality rates			
1. Total (i)	0.80	0.80	0.80
2. Fishing (p)	0.10	0.19	0.49
3. Natural causes (q)	0.70	0.61	0.31
Annual mortality rates			
1. Total (a)	0.55	0.55	0.55
2. Fishing (m)	0.10	0.17	0.39
3. Natural causes (n)	0.50	0.46	0.27

TABLE XI. Summary of catch and effort data for the commercial walleye fishery on Nipigon Bay, 1954–65. Each effort unit is based on 1 day's fishing.

Year	Gillnets			Poundnets			Total catch all gear (*lb*)
	Total catch (*lb*)	Total effort (*1000's of yards*)	Mean catch per 1000 yards (*lb*)	Total catch (*lb*)	Total effort (*no. of lifts*)	Mean catch per lift (*lb*)	
1954	5,240	94.3	55.6	12,252	50	245.0	17,492
1955	1,755	177.2	9.9	11,621	35	332.0	13,376
1956	4,375	328.5	13.3	1,092	31	35.2	5,467
1957	20,138	450.4	44.7	8,810	17	518.2	28,948
1958	11,709	361.1	32.4	23,527	52	452.4	35,236
1959	2,935	73.6	39.4	12,879	42	306.6	15,814
1960	3,594	93.7	38.4	4,652	16	290.7	8,246
1961	7,477	126.8	59.0	0	0	0	7,477
1962	4,887	76.6	63.8	672	13	51.7	5,559
1963	3,731	56.5	66.0	0	0	0	3,731
1964	3,675	140.0	26.2	178	8	22.2	3,853
1965	2,459	168.5	14.6	0	0	0	2,459

widely and are probably more indicative of the vagaries of the fishery and the seasonal movements of the fish than of any real indication of standing crop. Counts on the spawning grounds in 1956 indicated a larger spawning run in that year than in either 1955 or 1957. Catch per unit of effort and total catch for 1956, however, were extremely low for pound- and gillnets (Table XI), an indication that the fishermen probably missed the downstream run in late May and early June.

Annual expectation of natural death (v) is derived from the relationship $v = a - u$ (Ricker, 1958) and results in medium to high values (Table X). Instantaneous mortality rates were computed by using the series of equations presented in Ricker (1958, p. 25) and were used in estimating natural and fishing mortality rates. The instantaneous natural mortality rate (q) for 1957 was subsequently used in correcting population estimates for this factor.

The rate of decrease in natural mortality (n) is almost completely counterbalanced by a corresponding increase in fishing mortality rates. This condition would be likely to occur only if harvest took place immediately prior to or during that time of year when most of the natural mortality occurred. In the Nipigon Bay commercial fishery most of the catch was taken downstream from the effluence of the Nipigon River. By far the largest catch was taken in June (Table VI), immediately after the spawning run in the Nipigon River, and at a time after the greatest physiological stress on the mature fish during the year. Delayed natural mortality could be expected to occur in June as a result of spawning, but if these fish were harvested before actual mortality occurred, a close inverse relationship between fishing mortality rate and natural mortality rate would ensue. If exploitation and natural mortality occurred at a constant rate throught the year, the obvious relation between exploitation and natural mortality would not likely be as close.

POPULATION ESTIMATES

Two population estimates were computed for Nipigon Bay walleyes over 14 inches TL by the method of Parker (1955). The first estimated the number of walleyes on the spawning grounds in the Nipigon River in the spring of 1957; the second, the number of walleyes in Nipigon Bay as of September 1, 1957. The tagged fish used in the estimates were those marked prior to the fishing season in 1957, the year when exploitation rates were at a high level (0.34, Table X). The Parker method corrects for recruitment to the unmarked population after the end of the tagging period. As vulnerability corrections and survival rates were not precisely determined, corrections of the population estimates for vulnerability and mortality are approximate only. No harvesting of walleye took place for the first 24 days after tagging and the first computed ratio of marked to total fish in the catch (Table XII) was for the 25th day after tagging was completed.

Of the 504 walleyes which were tagged in the lower Nipigon River in 1957 (Table I) only 394 were used in computation (Table XII) after 11 fish less than 14 inches were subtracted from the total and the vulnerability factor

TABLE XII. Data from tagged and unmarked walleyes in the sport and commercial harvest used in regression method of Parker (1955) for population estimates.

Days after end of tagging	Total fish in sample	Tagged fish in catch / Total fish in catch	Arcsin $\sqrt{\text{ratio}}$	Tagged fish removed	Natural mortality of tagged fish	No. tagged fish at end of day
						394
25	129	0.0543	13.48	7	8	379
28	226	0.0133	6.63	3	1	375
29	1,783	0.0090	5.44	16	–	359
32	1,504	0.0100	5.74	15	–	344
33	1,998	0.0115	6.15	23	1	320
37	1,566	0.0038	3.53	6	1	313
39	714	0.0098	5.68	7	–	306
43	1,072	0.0093	5.53	10	2	294
47	563	0.0107	5.94	6	1	287
55	1,081	0.0139	6.78	15	2	270
64	1,786	0.0045	3.85	8	2	260
116	1,645	0.0079	5.10	13	11	236
	14,067	0.01772[a]		129	29	
		0.00575[b]				

[a]Ratio, as computed by regression, of tagged fish captured on 1st day to total fish captured.
[b]Ratio, as computed by regression, of tagged fish captured on 116th day to total fish captured.

of 0.8 was applied. The ratio, computed by regression, of the number of tagged fish in the catch on the first day after tagging was completed to the total number of fish in the catch on the 116th day after tagging was 0.01772 (Table XII). At this time 129 tagged fish had been removed from the population by harvesting. A recruitment-corrected estimate for the spawning population as of May 5, the end of the tagging period, was obtained by dividing the computed ratio for that date into the number of tagged fish extant (394), resulting in a rounded estimate of 22,000.

Similarly, an estimate was obtained for September 1, 1957, or 116 days after the end of the tagging period. By applying the instantaneous natural mortality rate (q = 0.31, Table X) on a daily basis to the number of tags extant at the end of the tagging period, the estimate for the number of tagged fish at large after a lapse of 116 days was obtained. The daily natural mortality rate, then, was 0.31/365 or 0.00084. The computed natural mortality of tagged fish at 116 days was 29; therefore the number of tagged fish extant at this time was 394 less (129 + 29) or 236. The regression-computed ratio for the 116th day is 0.00575, which when divided into 236 provided a population estimate of 41,000 for September 1, 1957.

The regression plotted from data in Table XII showed an aberrant point for the 25th day after tagging. If this point were removed from the regression,

a lower estimate of the spawning stock would result. However, as this aberrant point occurred on the first day of sampling, it likely represented a real value for the ratio of tagged fish in the catch to total fish in the catch as the mature river walleyes (high ratio marked) moved out into Nipigon Bay (low ratio marked).

The difference between the May 5 population estimate of 22,000 and the September 1 estimate of 41,000 may have been due to recruitment from three possible sources: immature fish over 14 inches TL; growth into the 14-inch length increment; immigration. As the May 5 estimate included only mature fish on the spawning grounds, substantial numbers of immature fish over 14 inches TL must have been present elsewhere in the Nipigon Bay watershed. In the 3 years of tagging in the lower Nipigon River, an immature walleye was never encountered. At a length of 14 inches, 75.0% of the males but only 28.6% of the females were mature (Table XIII). All males reached

TABLE XIII. Percentages of 185 male and 125 female walleyes from Nipigon Bay that were mature. Samples were taken far from the spawning grounds during the summer, fall, and winter, when a mixture of mature and immature fish could be expected.

Total length (*inches*):	12	13	14	15	16	17	18
Percentage mature males	0.0	37.5	75.0	100.0	–	–	–
Percentage mature females	–	0.0	28.6	66.7	90.5	96.0	100.0

maturity at 15 inches, but females not until 18 inches. Immature walleyes more than 14 inches long then, constituted a substantial portion of the total population. Recruitment by growth into the 14-inch length-class also probably contributed substantially to the population between May 5 and September 1, which included the greater part of the growing season. Recruitment by immigration from other populations was not indicated by the tagging data, and at best constituted only a small portion of the total recruitment.

The negative side of the balance sheet shows a rather high total mortality of 55% (Table X), which would decrease the effect of recruitment on the population estimates. Emigration from the population was represented by only 4 tagged walleyes captured in Black Bay out of a total of 373 recaptures from fish tagged in the Nipigon Bay watershed, or 1.1% of all tagged fish.

STATUS OF WALLEYE SPAWNING RUNS

Attempts were made to observe the walleye spawning run in the lower Nipigon River each May from 1955 to 1966 inclusive. Numbers of spawners were estimated each year for the shoreline areas that were most heavily

utilized. During the years 1955–58 the spawners were considered to be abundant; in 1959 and 1960 they were common, but not abundant enough to provide substantial numbers for tagging; in 1961 and especially in 1962 they were scarce; in 1963 only one walleye was observed on the spawning grounds; during the years 1964–66 inclusive, no walleyes were seen on the spawning grounds. As only one walleye spawning ground (Nipigon River) was located in the Nipigon Bay watershed, it is likely that abundance of adult walleyes there was indicative of population declines throughout the watershed.

During this same period a noticeable decline occurred in the numbers of spawning white suckers (*Catostomus commersoni*) and long-nosed suckers (*Catostomus catostomus*), which utilized the same spawning shoals as the walleyes. The suckers usually occurred on the spawning grounds concurrently with the walleyes but spawned after the walleye spawning was completed. No quantitative estimates of sucker decline were made, except that it was noted that a sucker spawning run has not occurred since 1964 and perhaps for several years previous. Both species of suckers, however, are still common in Nipigon Bay. It is probable that these fish utilize other spawning grounds than those in the Nipigon River.

EFFECTS OF SEA LAMPREYS

The sea lamprey has been blamed for the demise of the lake trout fishery in Lakes Huron and Michigan. (Hile, 1949; Hile et al., 1951; Fry, 1953). Hile and Buettner (1959) suggested that the sea lamprey may have been a most important factor in the decline of the walleye fishery in Saginaw Bay. They based this assumption on the similarity in timing of the failures of the walleye and lake trout fisheries, and on the summer–fall distribution of walleyes in relation to known sea lamprey movements.

In Nipigon Bay it is difficult to relate the timing of the lake trout decline with that of the walleye primarily because of the relatively low mean production of walleyes and the sharp fluctuations of abundance prior to the time of effective sea lamprey predation. The rapid decrease in walleye catches from 1959 through 1965 largely paralleled, however, the general downward trend of the lake trout fishery (Fig. 5A) for the same period. Minor fluctuations in both fisheries preclude the possibility of a close relation in the timing of the drops in abundance unless the reasons for these fluctuations can be determined.

All walleyes handled in the lower Nipigon River during the years 1955–57 were examined for sea lamprey wounds or scars. The highest wounding rate was observed in 1957 but was less than 1% of all walleyes examined, the data being as follows:

Year:	1955	1956	1957
No. of fish examined	225	1604	570
No. with wounds and/or scars	0	1	5
Percentage of fish with wounds and/or scars	0	0.06	0.9

These wounding rates would be significant only if a substantial portion of walleyes that are attacked by sea lampreys die shortly after. If this were the case an increase in natural mortality rate would be expected for the years 1955–57 coinciding with the increase in wounding for the same years. In actuality a decrease in natural mortality rates was computed for these 3 years (Table X).

An additional insight into the possible significance of sea lamprey predation is provided by comparing the Nipigon Bay and Black Bay walleye fisheries. Both bays have tributary streams which support substantial sea lamprey spawning runs. Presumably, the walleye populations in the two bays would be equally vulnerable to sea lamprey predation. As there was no noticeable decline in the Black Bay walleye fishery (Fig. 6) coinciding with the precipitous decline in Nipigon Bay (Fig. 5A), it is suspected that the sea amprey did not play a vital role in the latter decline.

EVIDENCE OF POLLUTION

Walleye dynamics in Nipigon Bay and contiguous waters cannot be adequately appraised without consideration of possible environmental changes and their effects. Foremost of these in Nipigon Bay is the artificial eutrophication caused from the effluent outfall of the kraft mill at Red Rock (Fig. 9). This mill achieved prominence in the late 1930's as a sulphite pulp mill with a capacity of 60 tons per day production. A changeover was effected in 1950 for kraft production at the rate of 390 tons per day. In 1955 the old kraft machines were partially converted to semi-chemical sulphite pulp production. By 1958 a bleach plant and new chemical recovery unit were installed in the mill. Present production (1966) of the kraft mill includes: 40 tons per day of semi-chemical sulphite pulp, 200 tons per day of groundwood, 120 tons per day soft kraft, 315 tons per day hard kraft, 400 tons per day kraft container board, and 200 tons per day newsprint (Anonymous, 1966–67). Apparently no marked changes in production rates have occurred since 1962. Effluent outfall has increased over the years but not necessarily proportional to the increase in production rates. In 1960 an average of 67 tons of suspended solids per day was dumped into Nipigon Bay at Red Rock (E. W. C. Turner, personal communication). A noted increase in both suspended and dissolved solids in the effluent outflow occurred between 1958 and 1962 (Ontario Water Resources Commission files), corresponding with the increased production rates during this period.

Of critical concern are the high levels of phenols found both in the effluent and throughout the west portion of Nipigon Bay. An Ontario Water Resources Commission effluent maximum for phenols in surface waters was exceeded by an average of 5 ppb at 11 stations in the western portion of Nipigon Bay, an indication that phenols and possibly other waste components are retained and concentrated within the bay. Although these concentrations of phenols are generally at subtoxic levels to fish (American Public Health Association, 1960) they can be responsible for imparting an unpleasant and

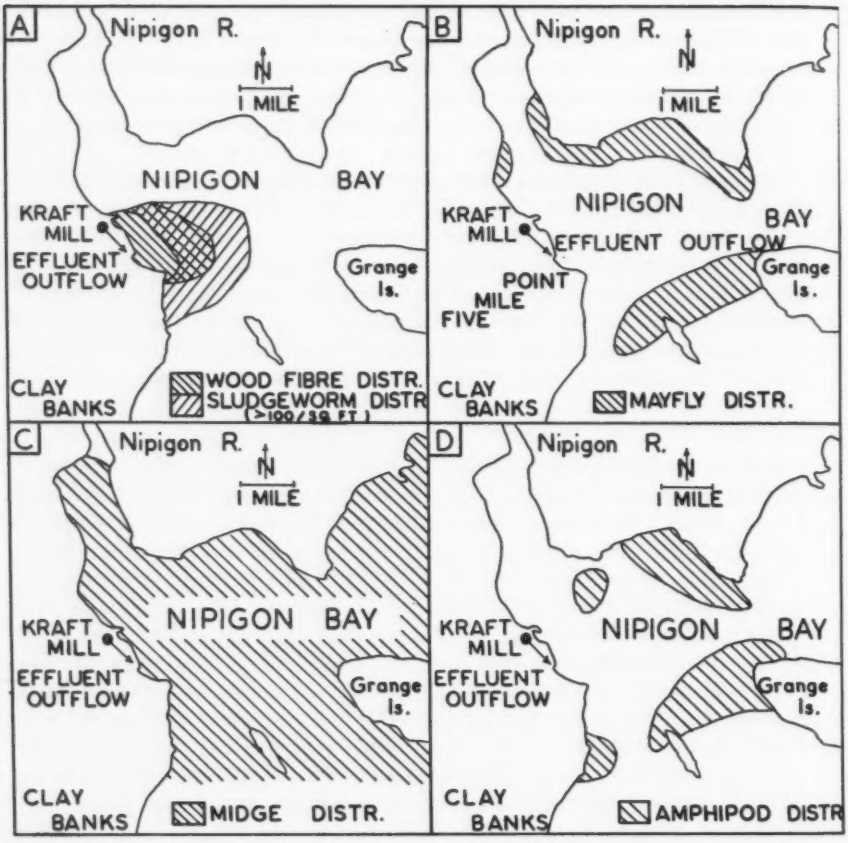

FIG. 9. Distribution (May 1966) on substrate of Nipigon Bay of (A) wood fibre and sludgeworms (*Limnodrilus hoffmeisteri* and *Tubifex tubifex*); (B) mayfly nymphs (*Hexagenia limbata*); (C) midge larvae (12 species); and (D) amphipods (*Pontoporeia affinis*); after German (MS, 1966).

obnoxious taste, thereby making fish unpalatable and consequently unmarketable. On July 22, 1961, Gordon Dampier, a commercial fisherman from Nipigon had a shipment of lake whitefish rejected by an American fish buyer because of unpleasant taste, resulting in an economic loss of $2500. Subsequently, fish buyers have rejected other shipments of fish (both lake whitefish and walleyes) originating from Nipigon Bay.

Flavour evaluations of fish flesh were carried out by the Ontario Water Resources Commission on fish taken from the vicinity of the kraft mill at Red Rock as well as other locations in Nipigon Bay. Of the 111 flesh samples taken near the mill, 92% had a foreign flavour and 71% possessed a strong flavour (M. J. German, personal communication). Broken down on the basis

of individual fish, a total of 80% taken near the mill possessed an objectionable flavour. The incidence of abnormal flavour was compared between control fish and fish from the kraft mill area using a Chi-square test (Snedecor, 1956). The difference in incidence of abnormal flavour between these two groups was highly significant ($P = 0.99$). Fish sampled in areas of Nipigon Bay remote from the kraft mill were untainted.

In the spring of 1966 a series of 35 stations were established in the west end of Nipigon Bay for the sampling of wood fibre and bottom organisms by means of a Peterson dredge covering sample areas of $9\frac{7}{8} \times 10\frac{1}{2}$ inches (German, MS, 1966). These samples were subsequently sifted through a 30-mesh-per-inch screened box and the macroinvertebrates preserved for later identification. Wood fibre deposits picked up in the dredge were greatest in a radius of about 1–$1\frac{1}{2}$ miles east and southeast of the effluent outflow (Fig. 9A).

Sludgeworms (Oligochaeta), which tolerate low dissolved oxygen concentrations related to organic pollution, are often used in pollution detection (Brinkhurst, 1962). Sludgeworms were abundant only in the vicinity of the kraft mill. *Limnodrilus hoffmeisteri* and *Tubifex tubifex* were present in densities greater than 100 per foot^2 for a radius of about 2 miles east and southeast of the effluent outflow (Fig. 9A). Oligochaetes were recorded at much lower densities at other locations within Nipigon Bay. Despite the relatively high concentrations of sludgeworms close to the kraft mill, very low densities were found immediately adjacent to the effluent outflow suggesting that the water there was toxic (Fig. 9A).

Mayfly nymphs (*Hexagenia limbata*) require dissolved oxygen concentrations greater than 1 ppm, and a suitably soft marl, mud, or clay substrate in waters less than 70 ft in depth (Hunt, 1953). The substrate requirements for *H. limbata* are met throughout the west end of Nipigon Bay, but three stations in the centre of the bay exceeded the depth requirement. Most noticeably, *H. limbata* was absent adjacent to the west shore of Nipigon Bay in the region of and downstream from the effluent outflow (Fig. 9B) where dissolved oxygen concentrations could be expected to be low and the substrate unsuitable due to wood fibre deposits and possibly dissolved toxic materials.

Midge larvae (Tendipedidae), which may be pollution-tolerant or facultative, occurred throughout Nipigon Bay (Fig. 9C). At least 12 species were present in the samples collected. Areas of least species diversity occurred in the central portion of the west end of Nipigon Bay, and along the western shore, including the area most exposed to the mill effluent where the facultative species did not exist (German, MS, 1966).

The amphipod *Pontoporeia affinis* had a pattern of distribution (Fig. 9D) closely similar to that of *H. limbata*. As *P. affinis* is favoured by deep waters (Cooper, 1962, p. 173), its absence from the central portion of the bay directly east and southeast of the effluent outflow is likely accounted for by organic pollution and sedimentation from the kraft mill.

The wood fibre accumulations, the concentrations of sludgeworms, the absence of mayfly nymphs and amphipods in the vicinity of and downstream

from the effluent outflow, and the reduction in number of species of midge larvae in the same area (Fig. 9) indicate severe organic pollution in the water between the effluent outflow and Five Mile Point, and moderate pollution within a $1\frac{1}{2}$ mile radius east and southeast of the discharge ditch.

GENERAL DISCUSSION

The failure in recent years of the commercial fishery in Nipigon Bay and a corresponding lack of success in the sport fishery in tributary inland waters suggest extermination of the walleye population. In 1966 the Ontario Water Resources Commission were unable to capture any walleyes in Nipigon Bay for taste tests (German, MS, 1966). The commercial catch that same year consisted of only seven walleyes. During 1967 no walleyes were captured by the commercial fishery in Nipigon Bay.

Three factors that possibly contributed to the demise of the walleyes in Nipigon Bay were: exploitation by the commercial and sport fisheries, sea lamprey predation, and an environmental change, namely pollution.

Commercial and sport fisheries, although capable of reducing a fishery under certain conditions to the limit of unprofitable exploitation, rarely exterminate a species, especially in a body of water the size of Nipigon Bay at relatively low exploitation rates. The complete absence of spawning walleyes from 1964 to the present, even when substantial but much reduced numbers were still evident in the commercial catch (about 4000 lb in 1964), suggests another causative factor. A much more intensive walleye fishery on Black Bay during the same period did not result in a noticeable decline in landed catch (Fig. 6). Gillnet effort in Nipigon Bay was greatly reduced after 1958 and trapnet lifts after 1960 (Table XI) but the walleye population did not respond. The sport fishery has been virtually nonexistent since 1963, about the same time that the adult walleyes disappeared from the spawning grounds. As the sport fishery was supported for the most part by walleyes which emigrated from Nipigon Bay to inland waters by early summer, it seems likely that some factor was preventing the ascent of these fish into the Nipigon River and eventually Polly Lake. As the commercial fishery did not operate until after the ice left Nipigon Bay, it did not create a barrier to walleyes moving into the Nipigon River to spawn or upstream into Polly Lake. The principal commercial catch was that segment of the population which spawned in the Nipigon River and then proceeded downstream into Nipigon Bay.

Predation by the sea lamprey does not provide a likely cause for the demise of the walleye population. The timing of the walleye decline was not coincident with the lake trout decline, a suggestion made by Hile and Buettner (1959) for the possible indication of sea lamprey predation on the walleye population in Saginaw Bay. The presence of sea lamprey wounds and scars never exceeded 1.0% of the adult walleyes sampled during the spawning runs in 1955–57. The sudden failure of the adult walleyes to return to the spawning grounds when substantial numbers still existed in Nipigon Bay suggested a factor other than sea lamprey predation as responsible for the decline. And

finally, the maintenance of the large Black Bay walleye population despite the presence of several favorable sea lamprey spawning streams tributary to Black Bay almost eliminates the sea lamprey from consideration.

Major environmental changes have been shown to take place in Nipigon Bay because of the kraft mill discharge. Some of these changes are likely due to cumulative effects dating back to the late 1930's. The accumulation of wood fibre on the substrate over the years has created an unfavourable bottom habitat for both walleyes and certain walleye food organisms (e.g. *H. limbata*) in the vicinity of, and downstream from, the mill to Five Mile Point. This is the area in which both the walleyes and the commercial fisheries were concentrated during 1955–57 as evidenced by the distribution of tag recaptures (Fig. 1).

Increases in dissolved solids were more subtle, but had an effect over a much greater area of Nipigon Bay as indicated by the phenol concentrations. The fact that some walleyes were captured with an objectionable taste indicated that at least some of the fish were not killed by the effluent. Although walleye movements are not known prior to spawning in the Nipigon River, it is likely that they ascended the main flow from the river up the west side of Nipigon Bay past Five Mile Point and the effluent outflow. Walleyes are noted for their positive rheotaxis especially at spawning time (Rawson, 1957) and would tend to orient themselves in the current and ascend to the river proper.

The importance of olfaction in certain salmonids in locating their spawning tributaries and individual spawning grounds is well documented (Hasler, 1966). If olfaction is also necessary for walleyes to locate their spawning grounds, then any dissolved foreign matter introduced into the stream below the spawning grounds could result in disorientation of the fish. This situation has previously been proposed as an explanation for decline in Lake Erie walleyes (Regier et al., 1968). The ultimate results of such a situation cannot be predicted but some possibilities are obvious. An avoidance reaction to the effluent may cause the walleyes to veer off course and deposit eggs on an unsuitable substrate, or fail to spawn and resorb their eggs. The latter occurrence has been observed for walleyes in other areas where certain environmental factors were unfavorable (Derback, 1947). High concentrations of total dissolved solids have been known to block the spawning migration of the anadromous striped bass, *Roccus saxatilis*, in California (Radtke and Turner, 1967). This occurrence could conceivably apply to the Nipigon Bay walleyes as some species of fish are known to be able to detect phenol concentrations as low as 0.5 ppb (Hasler and Wisby, 1950).

The effects on fish of pollution caused by effluents from sulphite, sulphate, and groundwood processes at pulp mills have been well documented (e.g. Höglund, 1961; Van Horn et al., 1950; Waldichuk, 1962). In a study on the Spanish River, Ontario, walleyes were one of the species most adversely affected by kraft mill wastes, which eliminated them for a 25-mile stretch below the mill outfall (Dymond and Delaporte, 1952). Paper-fibre sludge deposits in

the Rainy River, Minnesota, below pulp mills reduced survival of walleye eggs. Eggs deposited on the fibre sludge deposits were exposed to *Sphaerotilus* growths which prevented successful emergence of the fry (Smith and Kramer, 1963). Additional adverse effects of low oxygen and high hydrogen sulfide concentrations near the sludge-water interface were lethal to early life history stages of walleyes and some fish-food organisms (Colby and Smith, 1967).

Effluents at sublethal levels have been shown to inhibit the reproductive capacities of fish, interfere with certain physiological processes, retard growth and swimming speed, or impair fish taste, resulting in economic and aesthetic losses. MacLeod and Smith (1966) found that pulpwood fibre impaired oxygen uptake and swimming ability of fathead minnows, *Pimephales promelas*, especially at low oxygen concentrations. They suggested that the lowered active metabolism and lessened swimming endurance indicated stress conditions and would decrease survival and production of fish in natural habitats. Smith et al. (1965) found that suspended conifer groundwood was the most lethal fibre and had a greater effect on walleye fingerlings than on fathead minnows. Pulpwood fibre lowered resistance of these species to low oxygen concentrations and high water temperatures. Walleye fingerlings held in groundwood fibre suspensions grew slower, and had lower active metabolic rates, lower numbers of mucous cells on the gills, and a lower hematocrit (Smith et al., 1966). Impairment of fish taste by pulp mill effluents has also been observed (Baldwin et al., 1961).

Evidence of pollution effects on the walleye population in Nipigon Bay in the present study is obvious but largely circumstantial. The elimination of overexploitation and the sea lampreys as likely causative factors in the walleye decline adds weight to this evidence. It seems likely that the walleye has been exterminated from Nipigon Bay, with little hope for recovery until the major source of pollution is corrected.

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Rehabilitation of Lake Trout in the Apostle Islands Region of Lake Superior[1,2]

By William R. Dryer

U.S. Bureau of Commercial Fisheries
Biological Laboratory, Ann Arbor, Michigan

AND

George R. King

Wisconsin Department of Natural Resources
Division of Conservation, Bayfield, Wisc., U.S.A.

ABSTRACT

Marked success of rehabilitation of lake trout in Lake Superior has been due principally to the control of the sea lamprey and closure of the lake trout fishery in 1962 and large-scale plantings of yearling lake trout in 1959–66. After the sea lamprey became established in the late 1940s, spawning stocks of lake trout began to decrease and were almost nonexistent by 1960–61. After control of the sea lamprey and closure of the commercial fishery for lake trout in 1962, the abundance of spawning stocks began to rise and reached the highest levels on record in 1964–66. Successful spawning in 1964 and 1965 was demonstrated by catches of age-0 lake trout in 1965 and 1966, the first evidence of natural reproduction since 1959.

Plantings of hatchery-reared lake trout in Wisconsin waters of Lake Superior began in 1952. The percentage of hatchery-reared fish in catches of juvenile lake trout increased almost steadily from 1953 to 1965 (when nearly all were of hatchery origin). The abundance of juvenile fish increased from 1959 to 1962 and remained nearly constant in 1962–66. The success of lake trout plantings was highest in 1959–61 but generally declined after 1961; the success of the plantings was inversely related to the abundance of older lake trout.

Annual increments of growth of hatchery-reared lake trout varied from 1.1 to 5.0 inches after planting. The average lengths of fish of identical age-groups varied according to gear of capture, depth of water, and season. More than 65% of the season's growth of age-III lake trout took place after September.

The findings indicated that the present rate of stocking lake trout may be higher than necessary to maintain optimum abundance.

INTRODUCTION

Rehabilitation of lake trout (*Salvelinus namaycush*) in the Apostle Islands region of Lake Superior has been highly successful due to the control of the sea lamprey (*Petromyzon marinus*), closure of the commercial fishery for lake trout, and the large-scale plantings of yearling (14- to 20-month-old) lake trout by the State of Wisconsin.

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In 1962, after the 87% reduction in the catches of spawning sea lampreys at all electrical assessment barriers in Lake Superior (Great Lakes Fishery Commission, 1964) and the closure of the commercial fishery for lake trout, the Bureau of Commercial Fisheries and the Wisconsin Conservation Division increased their efforts to monitor changes in lake trout stocks. Information was needed on the proper size, season, and location for planting lake trout (Pycha and King, 1967), dispersal of the fish after planting (Pycha et al., 1965), efficient stocking rates, and the relative success of various hatchery plants. Needed also was information on the status of spawning stocks and evidence of natural reproduction.

This report describes the changes in spawning stocks, the effects of hatchery plantings on stocks of juvenile lake trout, the success of various plantings, the re-establishment of natural reproduction, and the growth of hatchery-reared lake trout.

MATERIALS AND METHODS

This study is based primarily on 15,651 lake trout taken in 1958–66 in experimental bottom trawls and small- and large-mesh gillnets fished by the Bureau of Commercial Fisheries research vessel *Siscowet* (Table I), and 12,381 lake trout caught in 4.5-inch mesh (extension measure) gillnets by Wisconsin commercial fishermen under special permit with the State. Records were also used of fish taken by the Wisconsin Conservation Division on the lake trout spawning grounds in 1951–60. All of the fish were taken in the Apostle Islands region of Lake Superior. Hatchery-reared lake trout were identified by fin marks used for the various plantings.

Sampling Gear and Fishing Methods

The bottom trawls were semiballoon with a 31-ft headrope, 2-inch mesh (extension measure) cotton body, and 0.5-inch mesh codend. Trawls were dragged along the bottom contour for 15 min at 3 miles per hour. A total of 557 tows were made, of which 344 were at four stations for systematic trawling.

The small-mesh nylon gillnets, 300 ft long and 6 ft deep, were fished in 12 net gangs. Each gang consisted of two nets of each mesh size from 2 to 3 inches, by 0.25-inch intervals, and 3.5-inch mesh. The nets were set along the bottom contours and fished for 48 hr. Nets of various meshes were randomly distributed within a gang. A total of 911,900 linear ft of small-mesh gillnets were fished, of which 621,800 ft were fished at three gillnet stations.

The gangs of large-mesh gillnets fished on the lake trout spawning grounds by the *Siscowet* usually consisted of 13 nets 275 ft long and had mesh sizes from 4.5 to 6 inches. The usual number of nets of the various mesh sizes fished in a gang was: 4.5-inch mesh, 2; 5-inch, 3; 5.5-inch, 4; and 6-inch, 4. Occasionally 150 ft each of 1.5- and 2.5-inch mesh nets were attached to the gang to collect smaller species suspected of preying on lake trout eggs. (Of the several hundred stomachs examined from longnose suckers, *Catostomus*

TABLE I. Numbers of juvenile lake trout captured with different gears by the R/V *Siscowet*, and percentages of hatchery origin (finclipped), in the Apostle Islands region of Lake Superior, 1958–66.

	Trawls			Small-mesh gillnets			Large-mesh gillnets		
Date	No. of tows	No. of fish	Percentage finclipped	Feet of net fished (*thousands*)	No. of fish	Percentage finclipped	Feet of net fished (*thousands*)	No. of fish	Percentage finclipped
1958	–	–	–	73.8	119	20.2	–	–	–
1959	58	73	55.3[a]	37.4	131	34.4	–	–	–
1960	66	85	75.4	148.5	181	33.7	–	–	–
1961	89	442	95.7	17.9	53	75.5	34.8	17	0.0
1962									
Spring	15	202	95.0	5.3	11	81.8	–	–	–
Summer	–	–	–	2.4	3	100.0	–	–	–
Fall	28	452	99.1	4.8	30	90.0	–	–	–
All seasons	43	654	98.2	12.5	44	88.6	57.4	100	0.0
1963									
Spring	48	654	98.9	23.0	268	91.4	–	–	–
Summer	–	–	–	41.6	440	94.3	–	–	–
Fall	32	440	99.5	38.8	390	90.0	–	–	–
All seasons	80	1094	99.1	103.4	1098	92.1	60.3	235	1.3
1964									
Spring	31	558	98.6	57.6	996	98.5	–	–	–
Summer	–	–	–	57.6	556	96.8	–	–	–
Fall	36	794	99.7	57.6	564	96.1	–	–	–
All seasons	67	1352	99.2	172.8	2116	97.4	17.6	626	3.9
1965									
Spring	40	440	99.1	57.6	695	97.3	–	–	–
Summer	–	–	–	57.6	574	98.6	–	–	–
Fall	37	601	98.7	57.6	678	97.2	–	–	–
All seasons	77	1041	98.9	172.8	1947	97.6	17.9	683	6.6
1966									
Spring	42	468	98.5	57.6	911	98.6	–	–	–
Summer	–	–	–	57.6	756	98.5	–	–	–
Fall	35	267	94.8	57.6	720	98.5	–	–	–
All seasons	77	735	97.2	172.8	2387	98.5	22.2	438	12.3
Total	557	5476	–	911.9	8076	–	210.2	2099	–

[a]Percentage based on 47 fish (26 were not examined for clipped fins).

catostomus, lake herring, *Coregonus artedi*, round whitefish, *Prosopium cylindraceum*, burbot, *Lota lota*, and lake northern chubs, *Hybopsis plumbea plumbea*, only one lake trout egg was found — in a longnose sucker.)

Gillnets fished by the Wisconsin Conservation Division on the spawning grounds in 1951–60 were of 5- to 7.5-inch mesh. The 6.5-inch mesh was most commonly used.

Seven "index" stations were selected for systematic fishing with trawls or small-mesh gillnets (Fig. 1). The criteria used in the selection of the stations included bottom type and topography, geographical location in relation to lake trout planting sites, and abundance of juvenile lake trout.

The four trawl stations were fished in the spring and fall, 1962–66, except that the Michigan Island station was fished only in the fall for native young-of-the-year lake trout. At least ten 15-min tows were made at each station during each season.

The three gillnet stations were fished in the spring, summer, and fall, 1963–66. Beginning in 1964, 21,600 ft of small-mesh gillnets (3600 ft of each mesh size) were fished during each season at the stations west of Madeline Island and south of Stockton Island, and 14,400 feet (2400 ft of each mesh size) were fished at the station east of Madeline Island. The total length of net of some mesh sizes was less in 1963 than in other years due to lack of gear.

As part of an interagency study to determine the survival of age-0 lake trout planted in the fall and age-I fish planted in the spring,[3] the Wisconsin Conservation Division in 1950 issued permits to five commercial fishermen to collect lake trout eggs in the Apostle Islands region for rearing in the Salmo Hatchery at Bayfield, Wisconsin. The annual collection of eggs continued under the State's supervision through 1960. Most of the fishing was on Gull Island Shoal, one of the major lake trout spawning areas in Wisconsin waters (Fig. 1). Egg collections by the State were discontinued after 1960 because the catches of spawning fish had become too small to provide the required number of eggs.

Beginning in 1961 the *Siscowet* fished Gull Island Shoal to assess changes in abundance of spawning lake trout. The number of sets on the shoal depended mainly on weather and abundance of spawning fish. The total length of large-mesh nets fished on the shoal in 1961–66 ranged from 17,600 ft in 1964 to 60,300 ft in 1963.

Collection and Analysis of Data

Lake trout caught in trawls and small-mesh gillnets were examined for clipped fins, measured (total length in inches), and returned to the water if alive. In 1962–65 the anal fin was removed from all lake trout returned to the water to determine the number of fish recaptured in later tows or in gillnet

[3]Returns of yearlings planted in the spring were 9–10 times greater than returns of fingerlings planted in the fall. Reviews of this study were given by Buettner (1961), Pycha et al. (1965), and Pycha and King (1967).

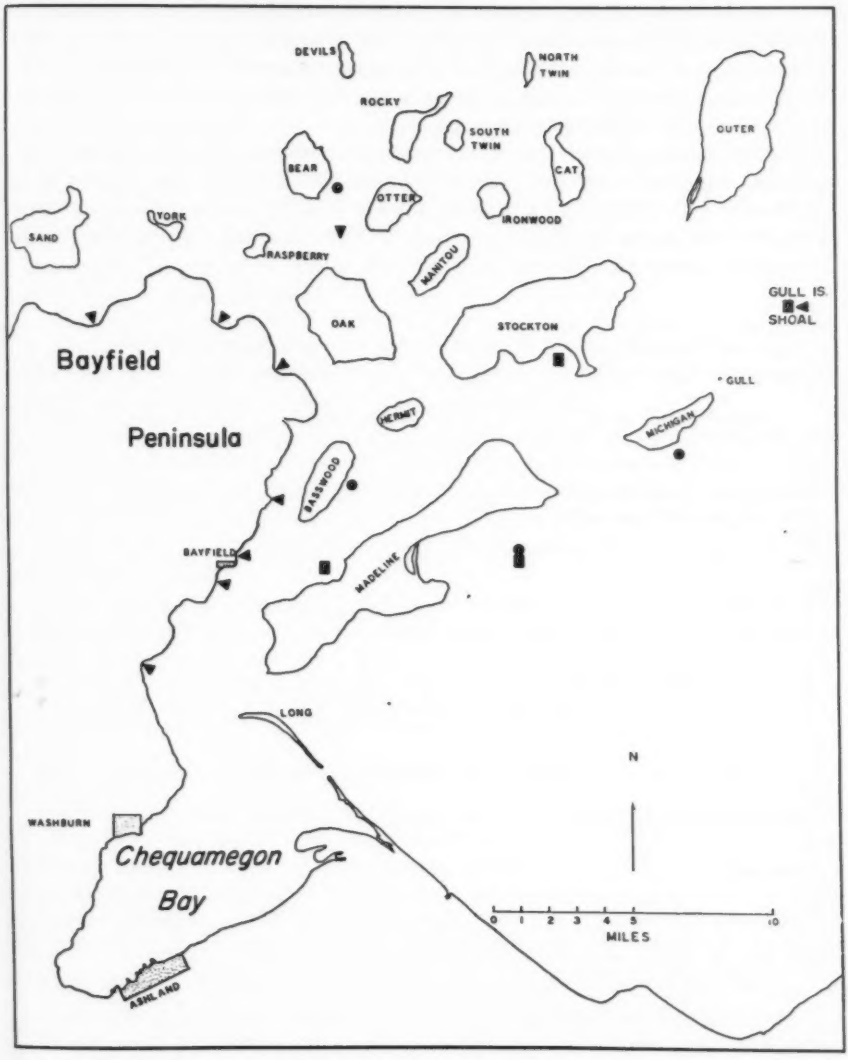

FIG. 1. Apostle Islands region of Lake Superior, showing index trawl stations (circles), index gillnet stations (squares), and lake trout planting sites (arrows).

sets. The number of anal-clip returns, however, was so small (less than 2%) that the practice was abandoned. The small return indicated either poor survival of the released fish or extremely high abundance of fish on the fishing grounds.

Information on lake trout from commercial gillnets was provided by the Wisconsin Conservation Division. The Division used its own vessel in 1962 and issued permits to three commercial fishermen in 1963–66 to fish a limited amount of gear at specified locations during April–May, August–September, and November–December.

Although finclipped lake trout have been planted in Wisconsin waters of Lake Superior every year since 1952 (except in 1957), the present study deals only with plantings in 1959–66 (Table II). The annual plantings varied from 151,000 fish in 1959 to more than 743,000 in 1964. The size of the fish at release ranged from 8.9 to 26.5 fish per pound.

TABLE II. Lake trout planted in the Apostle Islands region, 1959–66.

Year-class and mark	Date planted	No. planted	No. per pound
1958			
RPRM (right pectoral–right maxillary)	May 11–21, 1959	75,500	22.0
ARP (adipose–right pectoral)	"	75,500	21.8
1959			
LP (left pectoral)	May 11–13, 1960	161,350	20.0
1960			
A (adipose)	May 31–June 2, 1961	109,000	26.5
DRV (dorsal–right ventral	"	108,000	22.0
1961			
ABV (adipose–both ventrals)	May 7–31, 1962	255,500	17.7
DLP (dorsal–left pectoral)	June 14–26, 1962	120,800	22.5
DLV (dorsal–left ventral)	Sept. 24–Oct. 11, 1962	115,870	8.9
1962			
LPRV (left pectoral–right ventral)	Mar. 12–May 21, 1963	311,469	18.8
1963			
ARP (adipose–right pectoral)	Jan. 8–May 11, 1964	743,435	22.1
1964			
A (adipose)	Jan.–May, 1965	347,450	19.8
1965			
DLV (dorsal–left ventral)	Jan. 21–May 19, 1966	351,870	25.2

Some annual plantings during this period were omitted from the analyses because of unusually small returns or differences in planting locations. The 1962 fall planting of 115,870 fish, for example, was omitted from the trawl analysis because the fish were unusually large when planted and probably were not fully vulnerable to the trawl.

With the exception of the 1958 year-class, planted in 1959, all lake trout were planted from shore at various sites along the Bayfield Peninsula (Fig. 1). The 1959 releases were made by boat at the two offshore planting sites. The fish planted each year were divided about equally among the shore sites.

Estimates of the relative success of various hatchery plantings were based on the catch per unit of effort (CPE) for trawls and small-mesh gillnets at index stations and for commercial gillnets fished by cooperating commercial fishermen. The units of effort were 10 tows (15 min each) for trawls and 100,000 linear ft for small-mesh and commercial gillnets. Abundance indices on which we based our estimates were the catches (numbers of fish) per unit of effort adjusted through multiplication by the reciprocal of the size of the planting. To avoid small decimal values this figure was in turn multiplied by 10^5. Abundance indices were calculated individually for each plant at each station, season, and (for gillnets) by mesh size. The unweighted mean of seasonal values for each gear was used as the annual abundance index.

Estimates of abundance of fish on the spawning grounds was based on the number of fish caught per 10,000 ft of large-mesh gillnets. The number of feet of large-mesh nets fished on the spawning grounds was considerably less than that for small-mesh and commercial gillnets.

The study of growth of hatchery-reared lake trout was based on the average lengths at capture of fish of different age-groups collected in May 1962–66, before annual growth had resumed. Ages were assigned on the basis of fin marks (Table II) used for the various plantings. Although fin regeneration or faulty marking may have caused errors in age assignments, we believe that errors were negligible.

Age determinations of the native spawning-run lake trout were made from scales by the staff at the U.S. Bureau of Commercial Fisheries Biological Station at Ashland, Wisconsin. Although late onset of growth made age determinations difficult in some years, enough known-age (finclipped) fish were read correctly to give confidence in the age determinations for native fish.

CHANGES IN SPAWNING STOCKS

The major objective of the lake trout rehabilitation program on Lake Superior is to re-establish spawning stocks to a level capable of sustaining the population by natural reproduction. Early progress toward success of this objective was evidenced in Wisconsin waters in the fall of 1964 by a remarkable increase in the number of spawning fish, and in the summers of 1965–66 by the presence of naturally spawned (native) young of the year. Lake trout also reproduced successfully in the fall of 1965 and 1966.

Changes in Abundance

The abundance of spawning lake trout in the Apostle Islands region declined sharply from 1952 to 1953, rose slightly in 1955, and then declined almost steadily to 1960 when the catch per unit of effort (CPE) was only 3 males and 0.7 female per 10,000 ft of gillnets (Table III, Fig. 2). The CPE for males increased to 5 in 1961 but no females were captured. Abundance of spawning fish increased slowly in 1962–63 but in 1964 the CPE for males increased to the highest level since 1951 and that for females to the highest

TABLE III. Numbers of spawning lake trout captured per 10,000 ft of large-mesh gillnets on Gull Island Shoal, 1951–66, and of sea lampreys taken from electrical barriers on five streams tributary to Lake Superior.

Species	Year and (in parentheses) thousands of feet of gillnets lifted															
	1951 (60)[a]	1952 (28)	1953 (54)	1954 (60)[a]	1955 (60)	1956 (60)[a]	1957 (60)[a]	1958 (80)	1959 (105)	1960 (61)	1961 (35)	1962 (57)	1963 (60)	1964 (18)	1965 (18)	1966 (22)
Lake trout																
Males	68	94	11	16	42	26	24	29	20	3	5	16	34	310	341	154
Females	31	74	14	15	34	14	15	8	3	0.7	0	0.5	3	42	41	44
Sea lampreys (*thousands*)[b]	–	–	1.4	2.7	3.1	8.0	13.0	6.6	8.2	12.9	13.3	2.7	3.8	2.3	2.2	1.4

[a]Approximately 60,000 ft.

[b]Based on the annual catches of spawning sea lampreys at electrical barriers on the Betsy, Two Hearted, Sucker, and Miners rivers, and Furnace Creek. These were the only streams for which complete catch records were available for 1953–66.

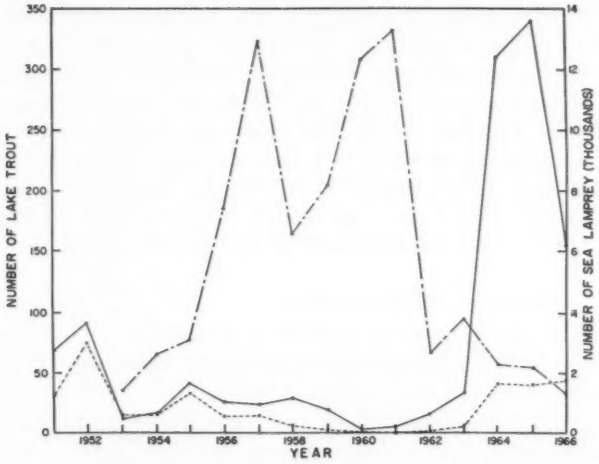

FIG. 2. Average numbers of spawning lake trout (males, solid line; females, short dashes) caught per 10,000 ft of gillnets fished on Gull Island Shoal, and numbers of adult sea lampreys (long and short dashes) taken at electrical barriers in five streams on the south shore of Lake Superior. (For names of streams and basis for selection, see Table III, footnote *b*.)

point since 1952 (310 males and 42 females per 10,000 ft of net). The CPE for males increased further in 1965 to 341 fish but dropped in 1966 to 154. The CPE for females remained about the same in 1964–66.

Age determinations revealed that native fish of year-classes 1957–59 were mainly responsible for the remarkable increase in spawning fish in 1964–65. Lack of recruitment to age-groups below VII contributed to the decline in abundance of males in 1966. The decline might also reflect the timing of the fishing in relation to the peak of spawning. A possibility exists that the peak of the spawning run was missed in 1966 and many of the males may have left the grounds before fishing began.

Although the mesh sizes fished in 1961–66 were slightly smaller and perhaps more efficient than those fished in 1951–60, the records show without question that spawning fish were practically nonexistent in 1960–61 and were abundant in 1964–66.

The changes in abundance of mature lake trout were inversely related to the abundance of sea lampreys in Lake Superior (Table III, Fig. 2). During the years of highest lamprey abundance (1956–61), stocks of spawning lake trout declined and reached the lowest level in 1961 when sea lamprey abundance was highest. After the 87% reduction in catches of sea lampreys at all 29 electrical barriers on Lake Superior (Great Lakes Fishery Commission, 1964) and the closure of the commercial lake trout fishery in U.S. waters of the lake in 1962, mature lake trout stocks began to increase.

Although little question exists that the sea lamprey was an important factor in the decline of spawning lake trout, other factors apparently contributed to the lower abundance in the early 1950's than in the middle 1960's. Abundance of sea lampreys in 1953 (the first year of record) was lower than in 1964–65 when spawning stocks were abundant. Had the sea lamprey been the only factor affecting abundance of mature lake trout, the size of spawning stocks in the early 1950's should have been as high as or higher than in 1964–66. The closure of the commercial fishery may have been largely responsible for the greater abundance in the middle 1960's.

Changes in Biological Characteristics

The decline and subsequent increase in abundance of lake trout on the spawning grounds were accompanied by changes in sex ratios, average lengths, and age distributions of the spawning stocks.

The percentage of females fluctuated between 31 and 56% in 1951–57 but began to decline in 1958 and reached zero in 1961 (Fig. 2). The percentages began an irregular increase in 1962 and reached 22% in 1966 — the highest since 1958.

The average lengths of spawning lake trout (Table IV) decreased with the decline in abundance and increased as the fish became more plentiful. The average lengths of the males decreased from 27.8 inches in 1957 to 25.4 inches in 1961, when abundance of the spawning stocks was lowest. As abundance began to increase in 1962 the average lengths of the males increased and reached 27.2 inches in 1966, the highest since 1957. Average lengths of the females followed similar trends; females were longest (32.4 inches) in 1957, declined to 28.0 inches in 1963, and increased to 29.4 inches in 1964 and 1966.

The age distributions of the spawning-run lake trout changed considerably over the period for which records are available (Table V). The most obvious change was the shift from old to young fish during the period of decline and the gradual increase in numbers of older fish during the years of recovery. Although no scales were collected from spawning lake trout in the Apostle Islands region before 1957, a sample taken near Marquette, Michigan, in 1953 (Rahrer, 1967), gives a general idea of the age composition of spawning-run lake trout before the sea lamprey became firmly established in Lake Superior (Table V). The mean ages of spawning lake trout were 9.8 for males and 10.4 for females. Most of the males were of ages IX and X and the females, X and XI. No fish younger than age VII were taken. In 1957 the mean age of Wisconsin fish was 8.6 for males and 10.0 for females. By 1961 the average age of the males had dropped to 6.0 (no females were taken) and in 1963 the mean ages were 6.7 for males and 7.5 for females. Most of the males were in age-groups VI and VII and none were older than IX. The presence of younger spawning fish may reflect faster growth, which can result in earlier maturity.

After 1963 the mean ages of the fish gradually increased and by 1966 the averages were 7.8 for males and 8.0 for females. The percentage contribution had declined for age-groups below VII and increased for age-groups above VIII.

TABLE IV. Length distributions of spawning-run lake trout from the Apostle Islands region, 1957–66. Fish were not measured in 1960; no females were captured in 1961.

Total length (*inches*)	Males									Females							
	1957	1958	1959	1961	1962	1963	1964	1965	1966	1957	1958	1959	1962	1963	1964	1965	1966
20.0–20.9	–	–	–	–	–	2	–	1	–	–	–	–	–	–	–	–	–
21.0–21.9	–	–	1	–	1	1	–	5	–	–	–	–	–	–	–	–	–
22.0–22.9	8	–	5	2	6	6	14	14	8	–	–	–	–	–	–	–	–
23.0–23.9	16	23	11	3	3	9	41	44	15	–	–	–	–	–	–	2	–
24.0–24.9	16	28	19	3	22	30	75	66	36	–	–	–	–	–	–	1	1
25.0–25.9	24	58	36	4	21	33	96	100	42	–	5	2	–	3	2	1	7
26.0–26.9	36	30	41	1	21	37	99	117	70	–	1	6	–	6	5	11	5
27.0–27.9	40	28	43	2	10	44	89	85	53	7	6	2	–	6	8	11	10
28.0–28.9	53	30	16	–	7	30	80	69	38	13	12	2	1	7	16	17	14
29.0–29.9	32	5	13	1	5	9	43	51	37	38	6	6	2	5	11	17	15
30.0–30.9	4	9	8	1	1	4	9	27	21	13	6	5	–	3	24	3	17
31.0–31.9	20	7	5	–	–	–	2	19	12	7	7	1	–	–	7	9	17
32.0–32.9	8	7	4	–	–	–	3	9	6	7	6	2	–	–	–	1	3
33.0–33.9	4	2	–	–	–	–	–	2	2	7	4	1	–	–	1	1	7
34.0–34.9	8	2	–	–	–	–	–	–	1	26	5	1	–	–	1	–	–
35.0–35.9	–	4	1	–	–	–	–	–	–	32	2	1		–	–	–	–
36.0–36.9	–	2	3	–	–	–	–	–	–	7	4	–	–	–	–	–	1
37.0–37.9	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–
38.0–38.9	–	–	–	–	–	–	–	–	–	7	2	–	–	–	–	–	–
Total	269	235	206	17	97	205	551	609	341	164	67	30	3	30	75	74	97
Avg length	27.8	26.5	26.5	25.4	25.9	26.4	26.5	26.8	27.2	32.4	30.5	29.1	29.2	28.0	29.4	28.6	29.4

TABLE V. Age compositions of spawning-run lake trout in Lake Superior off Marquette, Michigan, in 1953 and in the Apostle Islands region in 1957 and 1961–66.

Year and sex	No. of fish	Percentage in age-group										Avg age
		IV	V	VI	VII	VIII	IX	X	XI	XII	>XII	
1953[a]												
Males	142	–	–	–	0.7	2.2	29.6	44.4	18.5	4.4	–	9.8
Females	48	–	–	–	–	–	12.5	41.7	39.6	6.2	–	10.4
1957												
Males	269	–	–	–	17.9	35.7	32.1	7.1	–	3.6	3.6	8.6
Females	164	–	–	–	–	–	50.0	25.0	4.2	16.7	4.2	10.0
1961												
Males	17	–	27.6	58.6	6.9	3.4	3.4	–	–	–	–	6.0
Females	0	–	–	–	–	–	–	–	–	–	–	–
1962												
Males	97	4.2	18.9	29.5	37.9	8.4	1.1	–	–	–	–	6.3
Females	3	–	–	–	33.3	33.3	33.3	–	–	–	–	8.0
1963												
Males	205	–	8.8	32.2	36.6	21.5	1.0	–	–	–	–	6.7
Females	30	–	3.3	6.7	30.0	60.0	–	–	–	–	–	7.5
1964												
Males	551	–	4.3	32.4	50.0	10.9	2.4	–	–	–	–	6.7
Females	75	–	–	4.0	41.3	41.3	12.0	1.3	–	–	–	7.7
1965												
Males	609	–	5.1	29.7	33.5	19.5	11.0	1.0	0.2	–	–	7.1
Females	74	–	–	4.0	40.5	35.1	18.9	1.4	–	–	–	7.7
1966												
Males	341	–	1.5	7.6	30.5	36.1	17.6	6.5	0.3	–	–	7.8
Females	97	–	–	5.6	25.0	42.0	17.0	9.1	1.1	–	–	8.0

[a]Data from Rahrer (1967).

The changes in sex ratio, length, and age of the spawning lake trout were undoubtedly related to sea lamprey predation. The larger (and hence older) fish were most vulnerable to lamprey attacks and disappeared first. Numbers of females were réduced more rapidly than males, presumably because they mature at a greater size and age than males. After control of the sea lamprey in 1962, more young fish survived to maturity and more of the mature fish survived to spawn in subsequent years.

The 80% decrease in 1966 of age-groups V and VI (1961 and 1960 year-classes) gives evidence that spawning success was poor in 1959 and 1960. These observations were well supported by catches of native young-of-the-year lake trout by the *Siscowet*. Before 1965 the last young-of-the-year lake trout were taken in 1959, when three fish were captured off the west side of Outer Island (Fig. 1). Despite intensive trawling on grounds where age-0 lake trout were caught in 1953 or 1959 (the only years before 1960 during which trawling was carried out), none were captured in 1960–64.

Abundance of spawning stocks on Gull Island Shoal may decline unless hatchery-reared fish replace the weak or nonexistent year-classes of 1960–64 (Table III). It appears doubtful, however, that hatchery-reared lake trout will fully replace the native stocks. Evidence from fishing near the planting sites indicates that hatchery-reared lake trout spawn near the area of planting. In 1966, up to 75% of spawning lake trout from grounds near the planting sites were hatchery fish, whereas only 12% of the lake trout on Gull Island Shoal were hatchery reared.[4] The contribution of hatchery fish on Gull Island Shoal is too small to offset fully the lack of recruitment of younger native spawning lake trout.

The success of spawning in 1964 and 1965 was demonstrated by catches of young-of-the-year lake trout in 1965 and 1966. Bottom trawls fished in late summer and fall of 1965 captured 76 young of the year on grounds south of Michigan Island. In addition, four were taken on inshore grounds near the planting sites. The catch of 31 young-of-the-year lake trout at the Michigan Island station in 1966 gives evidence that spawning in 1965 was also successful.

CHANGES IN JUVENILE LAKE TROUT STOCKS

The percentage of hatchery-reared fish in catches of juvenile lake trout in the Apostle Islands region increased almost steadily from 1953 (the first year for which records are available) to 1965. Plantings of finclipped lake trout in Wisconsin waters of Lake Superior began in the spring of 1952 when 102,000 marked yearlings were released in the Apostle Islands region. Over 4.2 million finclipped lake trout were planted in Wisconsin waters in 1952–66.

Although the 1953–57 records are scanty and not entirely comparable with those of the present study, they provide a general idea of the buildup of hatchery fish. The Bureau of Commercial Fisheries R/V *Cisco* in 1953 captured 35 juvenile lake trout in the Apostle Islands region, of which 18 (51%) were finclipped. The first records for the *Siscowet* from this region came from gillnets fished in 1958 (Table I). Of 119 lake trout captured, 20% were of hatchery origin. The first dependable records from trawls fished by the *Siscowet* are for 1959 when 55% of 47 fish were hatchery reared. The percentage of hatchery-reared fish in the trawl catches climbed rapidly in the early 1960's and reached nearly 100% in 1965. The slight decline to 97% in 1966 was influenced by the catch of eight native yearling lake trout (1965 year-class).

Although the data from the *Siscowet* in 1959–61 are not strictly comparable with those of 1962–66, the records from trawls suggest that abundance of juvenile lake trout was considerably lower in the earlier than in the later period. The catch per 15-min tow was 1.3 in 1959 and 1960, and

[4]Since completion of this report we were informed by the staff of the Ashland Biological Station that the percentage of hatchery-reared lake trout on Gull Island Shoal in 1967 increased to 27%.

increased to 5.0 in 1961 and to 15.0 in 1962. The CPE did not change markedly in 1962–66. The numbers of fish caught per 15-min tow and per 1000 ft of small-mesh gillnets fished at index stations were:

Year:	1962	1963	1964	1965	1966
Trawls	15	14	20	14	9
Small-mesh gillnets	–	11	12	11	14

Because the fishing was only at index stations the catches would not necessarily measure changes in other areas.

SUCCESS OF HATCHERY PLANTINGS, 1959–66

In the computation of abundance indices for lake trout from different plantings made in 1959–66 (Table VI), numbers of fish caught per unit of effort were estimated for certain age-groups for which data were lacking. The estimates were based on the ratios of the average numbers of fish recaptured at different ages from all available data for a particular gear. Records from trawl catches, for example, indicated that the average number of lake trout from all plantings taken at age II was about 2.5 times the average number taken at age III. Missing data from trawls for certain groups at age II, therefore, were estimated to be 2.5 times the actual catch at age III. All other estimates were derived similarly.

The selectivity of the various gears affected the age composition of the catches. Trawls were most effective for hatchery fish of age-group II, small-mesh gillnets for the III group, and commercial gillnets for age-groups V and VI. Estimates of the relative success of the various plantings (Fig. 3), therefore, were based on the best represented age-groups from the various gears. The data for age-group V were used for commercial gillnets because they included a longer series than age-group VI.

Table VI and Fig. 3 give evidence that the relative success of lake trout planted in the Apostle Islands region has generally declined since 1961. The plantings of 1960 and 1961-A (1961 fish with adipose fin clip) were by far the most successful of all releases made in 1959–65. The success of the two plantings in 1959 and the 1961-DRV group was about average, but the success of the plantings in 1962–65 declined. Early returns in trawls of fish planted in 1966 were the lowest on record (Table VI).

The close agreement among the three types of sampling gear gives confidence in the reliability of the estimates of relative success for the various plantings. Agreement between small-mesh and commercial gillnets was close for all plantings (Fig. 3) but the trawl data for fish from the 1961-A release indicated a lower survival than indicated by the gillnet catches.

Possible Factors Affecting Success

Reasons for the decline in success of plantings after 1961 are not clear. The increased abundance of legal-sized (17.0 inches or longer) lake trout in

recent years may be a contributing factor. Analysis of the catch statistics for assessment fishermen in Wisconsin waters indicates that abundance of legal-sized lake trout has increased steadily since 1960. The abundance indices,[5] although strongly biased by low production in 1962–66 (production was limited to that needed for biological assessment), offer some indication of abundance of legal-sized lake trout during the period. The indices, given below, are expressed as percentages of the 1929–43 mean in adjacent Michigan statistical district S-2. Available data indicate that the quality of fishing in Wisconsin was very similar to that in district S-2.

Year:	1959	1960	1961	1962	1963	1964	1965	1966
Percentage of the 1929–43 mean	31	25	27	48	55	70	80	92

When abundance was extremely low in 1959–61 the success of the plantings was high but as abundance increased after 1961, the success of the releases declined. Competition for space and food, and predation by the larger lake trout may have reduced the survival of planted fish.

Comparisons between the number of fish in a planting and its relative success in later years (based on age-III fish from small-mesh gillnets) indicated that the large planting of 1962-ABV and the large releases of 1963–64 were less successful than the smaller plantings of 1959–61 (Table VI). The success of two nearly equal plantings of 1961, however, differed greatly and the small paired releases of 1959 were far less successful than the 1960 planting, which was more than twice as large.

Pycha and King (1967) found that the success of hatchery plantings of lake trout varied with the average size of the fish at planting. Groups of fish 21 per pound or larger were far more successful than those of smaller average size. The present study, however, showed no clear relation between the average size of the fish at planting and survival during the following 4 years. Of the five groups of fish larger than 21 per pound (Table II), only one (1960 planting) had better than average success, whereas the 1961-A release of relatively small fish (26.5 per pound) proved to be one of the most successful. Furthermore, the 1962-DLV fall planting of exceptionally large fish (8.9 per pound) experienced relatively poor success.

Pycha and King (1967) suggested that the high survival of the 1961-A release may have been the result of natural culling of the weaker fish by disease before planting. Buettner (1961) also found that lake trout which had suffered heavy mortality by disease before planting appeared to survive better than fish that suffered only average mortality in the hatchery.

The failure of the present study to show a relationship between survival and average size at planting may be due to the smaller differences in

[5]Abundance indices were computed by Richard L. Pycha (manuscript in preparation), Bureau of Commercial Fisheries Biological Station, Ashland, Wisconsin.

TABLE VI. Abundance indices, at different ages, of hatchery-reared lake trout from various plantings and (in parentheses) the average numbers caught per 10 trawl tows or per 100,000 ft of gillnets at index stations in the Apostle Islands region.

	Year of planting, mark,[a] and (in brackets) number planted												
	1959		1960	1961		1962			1963	1964	1965	1966	
Age-group	RPRM [75,500]	ARP [75,500]	LP [161,350]	A [109,000]	DRV [108,000]	ABV [255,500]	DLP [120,800]	DLV [115,870]	LPRV [311,469]	ARP [745,337]	A [347,450]	DLV [351,870]	Avg [231,470]
Trawls													
I	–	–	–	–	–	12.1	8.5	–	14.5	16.8	13.1	6.4	11.9
	–	–	–	–	–	(30.9)	(10.3)	–	(45.2)	(125.2)	(45.5)	(19.5)	(46.1)
II	29.0[b]	21.6[b]	40.9[b]	21.5	24.2	13.6	14.5	–	9.4	8.8	8.1	–	19.2
	(21.9)	(16.3)	(66.0)	(23.4)	(26.1)	(34.7)	(17.5)	–	(29.3)	(65.6)	(28.2)	–	(32.9)
III	11.4[b]	8.5[b]	16.1	8.7	7.0	6.5	6.5	–	3.1	4.4	–	–	8.0
	(8.6)	(6.4)	(26.0)	(9.5)	(7.6)	(16.6)	(7.9)	–	(9.7)	(32.8)	–	–	(13.9)
IV	3.1	2.3	2.6	2.2	1.7	1.8	2.7	–	2.0	–	–	–	2.3
	(2.3)	(1.7)	(4.2)	(2.4)	(1.8)	(4.6)	(3.3)	–	(6.2)	–	–	–	(3.3)
V	0.2	0.6	0.8	0.3	0.9	0.5	0.7	–	–	–	–	–	0.6
	(0.2)	(0.5)	(1.3)	(0.3)	(1.0)	(1.3)	(0.8)	–	–	–	–	–	(0.8)
Experimental gillnets													
I	–	–	–	–	–	–	–	–	1.8	3.0	2.6	1.8	2.3
	–	–	–	–	–	–	–	–	(5.6)	(22.4)	(9.0)	(6.3)	(10.8)
II	–	–	47.5[b]	53.5[b]	27.3[b]	15.3	4.4	21.7	27.5	20.3	21.0	–	26.5
	–	–	(76.6)	(58.3)	(29.5)	(39.1)	(5.3)	(25.1)	(85.7)	(151.3)	(73.0)	–	(60.4)

III	147.6[b]	152.7[b]	242.4[b]	272.9	139.2	133.1	81.8	65.9	92.2	81.5	99.1[b]	–	137.1
	(111.4)	(115.3)	(391.1)	(295.7)	(150.3)	(340.1)	(98.8)	(76.4)	(287.2)	(607.4)	(348.6)	–	(256.7)
IV	125.4[b]	129.6[b]	205.8	191.5	134.8	114.7	95.2	53.7	76.5	–	–	–	125.2
	(94.7)	(97.8)	(332.1)	(208.7)	(145.6)	(293.1)	(115.0)	(62.2)	(238.3)	–	–	–	(176.4)
V	59.2	61.2	96.6	56.4	59.5	80.6	50.0	32.4	–	–	–	–	62.0
	(44.7)	(46.2)	(155.9)	(61.5)	(64.3)	(205.9)	(60.4)	(37.5)	–	–	–	–	(84.6)
VI	15.7	14.7	23.7	26.7	27.4	–	–	–	–	–	–	–	21.6
	(11.9)	(11.1)	(38.2)	(29.1)	(29.6)	–	–	–	–	–	–	–	(24.0)
VII	3.7	3.7	10.7	–	–	–	–	–	–	–	–	–	6.0
	(2.8)	(2.8)	(17.3)	–	–	–	–	–	–	–	–	–	(7.6)
						Commercial gillnets							
III	–	–	–	20.4	7.1	–	–	–	–	–	–	–	13.8
	–	–	–	(22.2)	(7.7)	–	–	–	–	–	–	–	(15.0)
IV	–	–	80.9	89.5	28.9	18.4	6.6	21.6	13.5	–	–	–	37.1
	–	–	(130.5)	(97.6)	(31.2)	(47.0)	(8.0)	(25.0)	(42.0)	–	–	–	(54.5)
V	63.3	71.4	135.1	187.2	91.7	58.7	33.1	52.1	30.6[b]	–	–	–	80.4
	(47.8)	(53.9)	(218.0)	(204.0)	(99.0)	(150.0)	(40.0)	(60.4)	(95.3)	–	–	–	(107.6)
VI	62.3	72.8	156.8	185.3	134.3	185.3	–	–	–	–	–	–	132.8
	(47.0)	(55.0)	(253.0)	(202.0)	(145.0)	(473.4)	–	–	–	–	–	–	(195.9)
VII	47.7	53.0	110.3	–	–	–	–	–	–	–	–	–	70.3
	(36.0)	(40.0)	(178.0)	–	–	–	–	–	–	–	–	–	(84.7)
VIII	31.8	29.1	–	–	–	–	–	–	–	–	–	–	30.4
	(24.0)	(22.0)	–	–	–	–	–	–	–	–	–	–	(23.0)

[a]See Table II for identification of marks.
[b]Estimated by procedure explained in text.

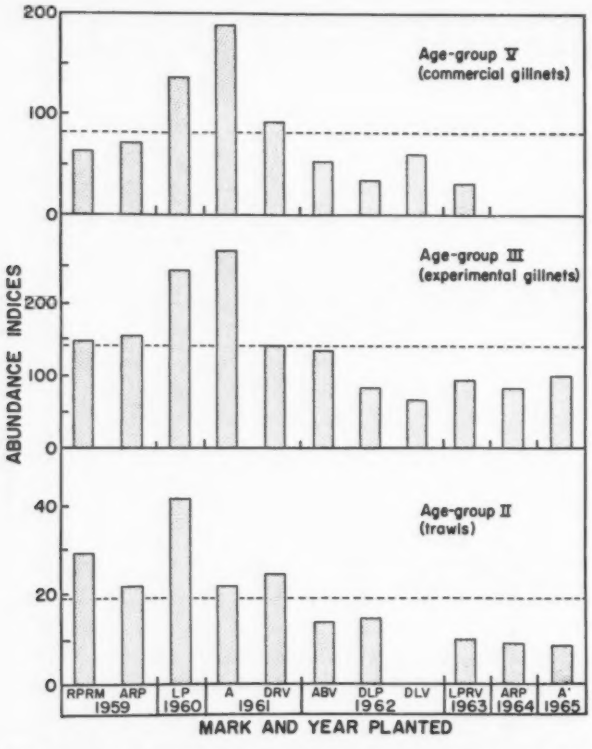

FIG. 3. The relative success of various lake trout plantings based on abundance indices for age-groups II, III, and V taken in three kinds of gear. The dotted horizontal lines represent the average success for all plantings. See Table II for identification of marks.

average size of the fish (8,9–26.5 fish per pound) than in Pycha and King's (1967) studies (2–80 per pound). All the plantings for which they rated success as "poor" consisted of fish which were smaller (34 or more per pound) than any of those considered here. Other factors affecting success of the plantings (e.g., increase in abundance of legal-sized lake trout, changes in the magnitude of the plantings) may have masked the small differences caused by size at release.

Another factor in the success of the plantings may have been differences in planting dates. The fish planted in 1959–62 were all released in May and June (except for the September–October release of 1962), whereas in 1963–66 a large portion of the fish were released under the ice in January–March (Table II). The fish planted in January–March averaged smaller than those planted the following May and June.

The decline in success of the more recent plantings and the failure of continued large-scale releases to raise total abundance of juvenile stocks suggest that the present rate of stocking in the Apostle Islands region may be neither economically sound nor desirable from the standpoint of rehabilitation.

Estimates of the success of hatchery plantings during the first few years after release may have practical application in forecasting returns to the fishery in later years. The average number of age-V lake trout caught per 100,000 ft of commercial gillnets, for example, was 3.79 times the mean number caught per 10 trawl tows at age II. By simple proportion, therefore, the number of fish expected from 100,000 ft of commercial gillnets at age V could be estimated to be 3.79 times the number taken per 10 trawl tows at age II. The dependability of such estimates would of course be affected by the amount of fishing effort and differences in mortality of various plantings between the ages of II and V. Forecasting the availability of lake trout to the fishery could be extremely valuable in the management of a closely controlled fishery.

GROWTH OF HATCHERY-REARED LAKE TROUT

Annual Growth in Length

Annual increments of growth of the hatchery-reared lake trout varied from 1.1 to 5.0 inches (Table VII) after planting in the Apostle Islands region of Lake Superior. The fish averaged 5.8 inches long when planted (age-group I) and were about 25 inches long after 8 years.

Growth was determined from the average lengths at capture of fish of the various age-groups taken in May 1962–66. The first year's growth was completed in the hatchery and was considerably greater than would be expected under natural conditions. This discussion deals only with growth of lake trout after they were released in the lake. The year classes were combined because differences in growth were negligible.

Sources of bias to estimates of growth included gear selectivity, wide ranges in length of the older age-groups, variations in average lengths according to depth of water, and an apparent decrease in length from spring to summer.

Gear selectivity was probably the most severe source of bias. The average lengths of fish of identical age-groups varied widely according to the gear of capture. For example, the average lengths of age-V fish taken in different gears were 12.9 inches for trawls, 15.6 inches for small-mesh gillnets (mean lengths ranged from 14.2 inches in the 2.25-inch mesh nets to 17.2 inches in the 3.5-inch mesh), and 20.1 inches for commercial (4.5-inch mesh) gillnets (Table VII, Fig. 4). The trawls tend to select the smaller members of age-groups above II, experimental gillnets probably select the larger individuals of age-groups II and III and the smaller members of groups VI and VII, and the commercial gillnets select the larger fish of age-groups III–VI and the smaller fish of the VIII group.

TABLE VII. Length distributions of age-groups of hatchery-reared lake trout collected in trawls in May 1962–66 and in small- and large-mesh gillnets in May 1963–66 (year-classes combined). T = trawls; S = small-mesh gillnets; L = large-mesh gillnets.

Total length (*inches*)	I	II		III			IV			V			VI		VII		VIII
	T	T	S	T	S	L	T	S	L	T	S	L	S	L	S	L	L
3.0–3.9	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4.0–4.9	59	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5.0–5.9	207	5	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
6.0–6.9	164	110	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7.0–7.9	28	465	33	6	2	1	–	–	–	–	–	–	–	–	–	–	–
8.0–8.9	1	428	37	63	5	–	–	–	–	–	–	–	–	–	–	–	–
9.0–9.9	–	110	17	204	94	1	13	4	1	1	1	–	–	–	–	–	–
10.0–10.9	–	16	15	217	301	15	19	21	1	1	4	–	–	–	–	–	–
11.0–11.9	–	–	3	87	224	13	52	140	5	4	8	–	–	–	–	–	–
12.0–12.9	–	–	3	16	79	7	41	229	11	9	38	2	1	–	–	–	–
13.0–13.9	–	1	–	2	35	14	26	192	15	7	78	4	7	–	–	–	–
14.0–14.9	–	–	–	–	20	9	2	152	22	4	126	12	8	3	1	1	–
15.0–15.9	–	–	–	–	6	8	2	83	25	1	114	17	15	3	2	–	–
16.0–16.9	–	–	–	–	1	6	–	40	36	–	80	22	13	5	1	–	–
17.0–17.9	–	–	–	–	–	6	–	14	160	1	48	85	18	21	1	1	–
18.0–18.9	–	–	–	–	–	1	–	5	231	–	30	214	14	45	1	1	–
19.0–19.9	–	–	–	–	–	3	–	1	222	–	20	363	7	134	2	9	1
20.0–20.9	–	–	–	–	–	–	–	1	104	–	7	347	15	203	2	17	3
21.0–21.9	–	–	–	–	–	1	–	1	45	–	10	242	14	227	5	37	2
22.0–22.9	–	–	–	–	–	–	–	–	12	–	9	126	7	185	6	33	9
23.0–23.9	–	–	–	–	–	–	–	–	4	–	–	67	4	130	3	22	5
24.0–24.9	–	–	–	–	–	–	–	–	–	–	–	20	4	63	3	29	6
25.0–25.9	–	–	–	–	–	–	–	–	2	–	–	2	2	33	1	24	13
26.0–26.9	–	–	–	–	–	–	–	–	–	–	–	3	–	21	–	8	4
27.0–27.9	–	–	–	–	–	–	–	–	–	–	–	–	–	7	1	2	4
28.0–28.9	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	2	5
29.0–29.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
30.0–30.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
31.0–31.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
No. of fish	463	1136	113	596	767	85	155	883	896	28	573	1526	129	1082	29	351	54
Avg length	5.8	8.0	8.7	10.1	11.1	13.6	11.8	13.4	18.6	12.9	15.6	20.1	18.5	21.7	21.3	22.9	24.9

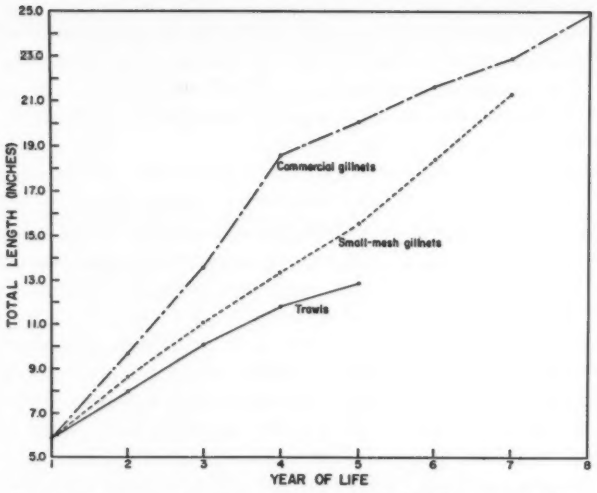

FIG. 4. Average lengths at capture of hatchery-reared lake trout of different ages, taken in three kinds of gear.

Estimates of growth were further complicated by the wide range in length of the older age-groups. The range in length of age-I fish when planted (Table VIII) was only 4.7 inches (from 3.9 to 8.6 inches), but at age V the ranges

TABLE VIII. Average total lengths (inches) of various age-groups of hatchery-reared lake trout from trawls fished at different depths in the Apostle Islands region, June 1962–63. Number of fish in parentheses.

Date of collection and depth (*fath*)	Age-group and fin mark[a]					
	I	II	II	III	III	IV
June 1962	ABV	A	DRV	LP		
15–19	–	8.0	7.8	11.7	–	–
	–	(12)	(5)	(5)	–	–
20–29	6.2	7.6	7.8	10.1	–	–
	(27)	(97)	(76)	(90)	–	–
30–34	6.2	7.9	7.7	9.8	–	–
	(8)	(16)	(31)	(17)	–	–
June 1963	LPRV	ABV		A	DRV	LP
15–19	5.7	7.9	–	11.6	10.6	12.4
	(10)	(8)	–	(15)	(6)	(9)
20–29	5.9	8.0	–	10.5	9.9	11.8
	(40)	(275)	–	(110)	(62)	(60)

[a]See Table II for identification of marks.

widened considerably and varied according to the type of gear in which they were captured. Age-group V fish were from 9.9 to 17.6 inches long in trawls, from 9.9 to 22.7 inches in small-mesh gillnets, and from 12.0 to 26.9 inches in commercial gillnets. The length range of age-group V lake trout, therefore, was 17.0 inches (from 9.9 to 26.9 inches).

The average lengths of hatchery-reared lake trout varied also according to depth of water. Records of lake trout from trawls fished in June 1962–63 by the *Siscowet* in the Apostle Islands region indicated that the average lengths of fish of age-groups I and II did not decrease with depth but that fish of age-groups III and IV were without exception larger in shallow water (Table VIII). Records from experimental gillnets (not given here) indicated that the average lengths of fish of age-groups II–V decreased with depth of water during all seasons. An inverse relation between depth of capture and average length of lake trout (all age-groups combined) was demonstrated by catches of the R/V *Kaho* in Keweenaw Bay, Lake Superior, in 1964–65 (Table IX). Mean length decreased steadily with increasing depth of water. Fish from depths of less than 10 fath averaged 7–10 inches longer than those from depths greater than 39 fath.

TABLE IX. Average total lengths (inches) of lake trout taken in trawls by the R/V *Kaho* from various depths in Keweenaw Bay, Lake Superior, 1964–65. Numbers of fish in parentheses.

Depth (*fath*)	1964		1965		Samples combined[a]
	Spring	Fall	Spring	Fall	
<10	17.1	20.5	18.9	–	18.8
	(20)	(5)	(12)	–	(37)
10–19	14.8	18.3	20.4	16.5	17.5
	(19)	(12)	(20)	(17)	(68)
20–29	11.8	17.3	19.3	15.7	16.0
	(138)	(22)	(43)	(51)	(254)
30–39	8.7	11.5	12.5	10.4	10.8
	(407)	(89)	(190)	(134)	(820)
>39	9.1	10.5	11.7	–	10.4
	(76)	(87)	(37)	–	(200)

[a]Unweighted mean.

The presence of larger lake trout in shallow water may be due to changes in food preferences with increased size of fish. Dryer et al. (1965) found that the importance of fish in the diet of lake trout increased as the lake trout grew larger. As the larger members of an age-group become more dependent on fish for food they may migrate to shallow water, where small forage species (mainly American smelt, *Osmerus mordax*) are more abundant. Fish of age-groups I and II and the smaller members of age-groups III–V depend mostly on crustaceans for food and may therefore remain in deeper water (Dryer, 1966).

The average lengths of fish of certain age-groups taken from the same depth with the same gear decreased from spring to summer. Almost without exception the average lengths of lake trout of age-groups III–V from small-mesh gillnets fished at 21–26 fath were shorter in July than in May of the same year (Table X).

TABLE X. Average lengths (inches) of lake trout from small-mesh gillnets fished at depths of 21–26 fath in May and July 1963–66. Number of fish in parentheses.

	Year-class, fin mark,[a] and age-group								
Year and month of capture	1959	1960		1961			1962	1963	1964
	LP	DRV	A	ABV	DLP	DLV	LPRV	ARP	A
1963	IV	III	III	II					
May	13.7	11.5	12.2	8.4	–	–	–	–	–
	(27)	(6)	(18)	(2)	–	–	–	–	–
July	13.3	11.3	11.8	9.5	–	–	–	–	–
	(102)	(55)	(104)	(9)	–	–	–	–	–
1964	V	IV	IV	III	III	III	II	–	–
May	15.7	13.6	14.1	11.0	10.5	11.5	9.2	–	–
	(159)	(132)	(236)	(225)	(55)	(69)	(21)	–	–
July	13.8	12.4	13.2	11.0	10.4	11.1	9.1	–	–
	(31)	(47)	(44)	(225)	(61)	(25)	(51)	–	–
1965	VI	V	V	IV	IV	IV	III	II	–
May	18.3	15.6	16.6	13.0	12.7	13.2	11.2	8.2	–
	(42)	(55)	(57)	(189)	(63)	(50)	(133)	(53)	–
July	19.3	14.8	14.6	12.9	12.2	12.6	11.1	8.7	–
	(8)	(26)	(23)	(142)	(67)	(25)	(159)	(89)	–
1966	VII	VI	VI	V	V	V	IV	III	II
May	20.3	18.2	19.0	15.1	14.5	16.0	13.2	11.0	9.0
	(21)	(29)	(36)	(202)	(57)	(32)	(186)	(261)	(33)
July	24.7	17.9	19.6	14.2	13.5	13.9	12.6	10.9	9.1
	(4)	(8)	(5)	(66)	(27)	(16)	(135)	(411)	(40)

[a]See Table II for identification of marks.

The length distributions of age-IV and age-V lake trout taken in May and July (Table XI) give evidence that the larger members of an age-group were poorly represented on the fishing grounds in July. The percentage of age-V lake trout longer than 15.4 inches dropped from 37.1% in May to 12.1% in July; age-IV, from 6.5% in May to nil in July.

TABLE XI. Length distributions of two age-groups of lake trout from small-mesh gillnets fished at 24–26 fath in May and July 1966.

Total length (*inches*)	Age-group, fin mark,[a] and month of collection			
	IV — LPRV		V — ABV	
	May	July	May	July
9.5–9.9	–	1	–	–
10.0–10.4	–	1	1	–
10.5–10.9	2	5	1	1
11.0–11.4	11	6	1	–
11.5–11.9	22	17	1	2
12.0–12.4	28	31	5	3
12.5–12.9	30	34	8	3
13.0–13.4	28	16	14	9
13.5–13.9	18	10	24	9
14.0–14.4	12	10	33	9
14.5–14.9	14	3	21	8
15.0–15.4	9	1	18	14
15.5–15.9	6	–	21	4
16.0–16.4	1	–	15	3
16.5–16.9	3	–	11	–
17.0–17.4	1	–	4	1
17.5–17.9	1	–	7	–
18.0–18.4	–	–	4	–
18.5–18.9	–	–	2	–
19.0–19.4	–	–	4	–
19.5–19.9	–	–	3	–
20.0–20.4	–	–	–	–
20.5–20.9	–	–	2	–
21.0–21.4	–	–	2	–
Total	186	135	202	66
Avg length	13.2	12.6	15.1	14.2
Percentage over 15.4 inches	6.5	0.0	37.1	12.1

[a]See Table II for identification of marks.

The apparent decrease in length from spring to summer, like the decrease in length with increasing water depth mentioned above, may be due to changes in food preferences. The larger members of an age-group may migrate to shallow water some time after May in search of small forage fish. Dryer (1966) found some evidence that lake trout moved from deep to shallow water from spring to fall.

Because of the wide variation in average lengths of fish of identical age-groups from the various gears (Table VII, Fig. 4) and because the catches from trawls and gillnets cannot be combined on the basis of common units of effort, sound estimates of general growth are impossible. Annual increments

of growth of lake trout from trawls decreased from 2.2 inches in the 2nd year of life to 1.1 inches in the 5th. The fish were 12.9 inches long at the end of 5 years. Lake trout from small-mesh gillnets grew 2.9 inches in their 2nd year of life, 2.9 in their 6th, and 2.8 inches in their 7th. The fish were 21.3 inches long at the end of 7 years. The annual increments of fish from commercial gillnets ranged from 5.0 inches in the 4th year to 1.2 inches in the 7th year of life. The fish were 24.9 inches long at the end of 8 years.

Native lake trout collected in trawls and small-mesh gillnets from Lake Superior in 1952–53 grew 3.9 inches in the 1st year of life (compared with 5.8 inches for hatchery-reared fish), were 6.7 inches long at the end of the 2nd year, and reached 8.6 inches after 3 years (Eschmeyer, 1956). The 2nd- and 3rd-year increments were 2.8 and 1.9 inches, respectively, compared with 2.2 and 2.1 inches for hatchery-reared lake trout from trawls in the Apostle Islands region in the present study.

Seasonal Growth

Seasonal growth of hatchery-reared lake trout was determined from the average lengths of fish from the 1962-ABV planting during various time periods in 1962–66. This was the only group for which complete records were available for ages I through V.

The records from trawls suggest that the fish grew at a nearly uniform rate during their 2nd and 3rd years of life (Fig. 5). The average length of the age-I fish (2nd year of life) in early June was the same (5.8 inches) as

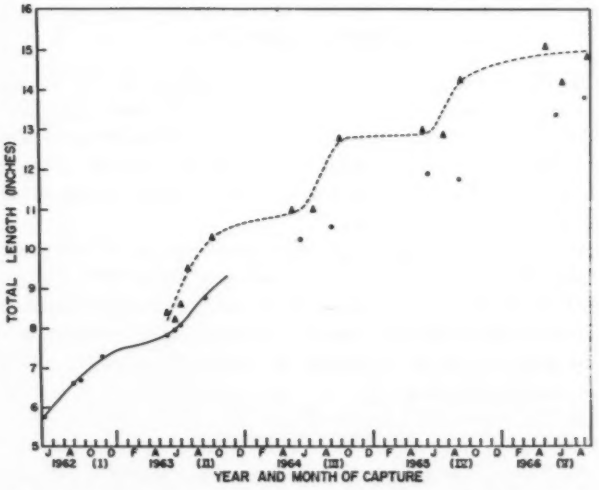

Fig. 5. Average lengths of lake trout recaptured from the 1962-ABV planting in trawls (circles) and experimental gillnets (triangles) for various dates, 1962–66. The age-groups are in parentheses after the year of capture. The curves were drawn by inspection.

that of the fish planted in May. The fish had grown 0.8 inch by early September and 1.5 inches by the end of November.

Late fall or winter growth was evidenced by the increase in average length from 7.3 inches in late November 1962 to 7.8 inches the following May. Third-year growth of lake trout from trawls was 2.3 inches (from June 1963 to June 1964). Over 65% of the growth (1.5 inches) took place after mid-September 1963. The curve for fish from trawls (Fig. 5) was not extended beyond the 3rd year of life because samples were collected only in June and September in the later years.

Lake trout from small-mesh gillnets appeared to grow rapidly in late summer and fall (Fig. 5). Fourth- and 5th-year growth did not begin before August but by late September the fish had grown well over 1 inch. The 5th-year growth (from May 1965 to May 1966) was 2.1 inches, of which 0.8 inch (38%) took place after September,

The apparent difference in seasonal growth of lake trout from trawls and small-mesh gillnets may be influenced by the migration in early summer of larger members of an age-group to shallow water. If the larger fish left the vicinity of the index stations in early summer or throughout the summer, estimates of growth based on the average lengths of the remaining smaller fish are not representative of the total population.

Eschmeyer (1956) suggested that lake trout of ages 0 and I in Lake Superior continue to grow to or beyond the end of the calendar year. Rahrer (1967) found that lake trout of ages I and II in Lake Superior had completed only 63% of their annual growth by October and fewer than 30% of ages VII–XII had started growing by October.

FUTURE PROSPECTS

The remarkable recovery of lake trout stocks in the Apostle Islands region makes the outlook for complete success of lake trout rehabilitation appear excellent. Natural reproduction, which has already been demonstrated, should soon replace hatchery plantings. The percentage of hatchery fish among juvenile stocks began to decline in 1966 and the trend should continue with the buildup of native stocks.

The decline in the success of hatchery plantings since 1961 and the failure of juvenile lake trout to increase in abundance since 1962 suggest that the present rate of stocking may be larger than is necessary to maintain optimum abundance. Hatchery-reared lake trout in excess of those needed in the Apostle Islands region might well be diverted to other areas where the progress of rehabilitation has been slow.

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Recurrent Group Analysis of Species Assemblages of Demersal Fish in the Gulf of Guinea[1]

By E. W. Fager

Scripps Institution of Oceanography, La Jolla, Calif., U.S.A.

AND

A. R. Longhurst

U.S. Bureau of Commercial Fisheries
Fishery-Oceanography Center, La Jolla, Calif.

ABSTRACT

Recurrent species groups were formed by computer analysis from the data on occurrence of demersal fish at 480 trawl stations in the tropical eastern Atlantic; these recurrent groups were then marshalled into a small number of major species assemblages, or communities in the wide sense, on the basis of intergroup relationships. These assemblages proved to be remarkably similar in composition and ecology to the communities of demersal fish recognized earlier by a number of authors from their subjective analysis of trawling survey data in this region. The computer analysis provided finer detail on both the structure and distribution of the assemblages and their constituent groups. This confirmation of the reality of the subjectively recognized communities suggests (1) that the recognition of such assemblages by subjective methods has a place in the assessment of multispecies fish resources but (2) that where the method is practicable, the machine processing of recurrent species groups in the analysis of trawl survey data can be a valuable tool in resource evaluation.

INTRODUCTION

During the last two decades many demersal trawl surveys have been performed by fishery agencies in West Africa to assess the fishery resources of the tropical continental shelf. It became evident during this period of exploration that not only was there a homogeneous fauna of demersal fish the whole length of the tropical Gulf of Guinea but also that there was a considerable degree of agreement among the various authors who had written about it as to how this fauna was organized into recurrent species groups or communities. This agreement is found in the descriptions of the demersal fauna in all 10 of the surveys that were reported in such a manner that their results could be used to identify recurrent species groupings of demersal fish (Table I), although (as can been seen from the tabulation) there was little uniformity in the descriptive terms employed. The species assemblages may be briefly described as follows:

i — Two rather similar assemblages (A and B in Table I), each dominated by species of Sciaenidae, Polynemidae, Ariidae, Cynoglossidae, Pomadasyidae,

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TABLE I. The species assemblages of demersal fish recognised subjectively in the Gulf of Guinea, with the terminology used by each author. (A plus (+) sign indicates that an author recognized an assemblage without applying a descriptive term to it.)

Area and author	Assemblage of demersal fish				
	A	B	C	D	E
Gambia (Longhurst, 1963)	Sciaenid fauna		Sparid fauna		Deep fauna
Guinea (Postel, 1955)	+		+		
Sierra Leone (Longhurst, 1963)	Sciaenid fauna		Sparid fauna		Deep fauna
Ghana (Salzen, 1957)[a]	+		+		+
Ivory Coast (Lassarat, 1957)	+				
Togo and Dahomey (Crosnier and Berrit, 1966)	Peuplement des fonds vasa-sableux baignées par des eaux chaudes	Peuplement des fonds durs baignées par eaux chaudes and Peuplement fonds sableux eaux chaudes		Peuplement des fonds vaseux baignées eaux froides	
Nigeria (Longhurst, 1963)	Offshore sciaenid sub-community	Estuarine sciaenid sub-community	Supra-thermocline sparid community	Sub-thermocline sparid community	Deep community
Cameroon (Crosnier, 1964)	Peuplement des fonds vaso-sableux baignées eaux chaudes		Peuplement des fonds sableux eaux chaudes	Peuplements des fonds vaseux eaux froides	
Congo (Poll, 1951)	Faune côtière			Faune de bordure continentale	Faune de la pente continentale
Congo (Durand, 1966)	Peuplement littoral			Peuplement de bordure continentale	Peuplement du talus continentale

[a]Salzen (1957) also recognized an assemblage (F) at about 400 m and deeper, on the upper continental slope.

Drepanidae, and Clupeidae. These assemblages, one of which occurs on the continental shelf (A) and the other in estuaries (B), differ mainly in the substitution of some closely related species, and in differences in the relative

abundance and frequency of some species and genera, in the two types of habitat.

ii — Two assemblages dominated by Sparidae and Denticidae and including a number of species of other families typical of sandy deposits, such as Triglidae, Mullidae, Synodidae, Scorpaenidae, Platycephalidae, and others. The two assemblages were sometimes confused and appear as C and D in Table I. They have different depth distributions, the one (C) above and the other (D) below the thermocline.

iii — An assemblage (E) occurring below the break of slope of the continental shelf at about 60–80 m and extending to 200–300 m depth. Many of the taxa are also found in similar assemblages at similar depths off western Europe. The main genera in this assemblage are *Chlorophthalmus*, *Peristedion*, *Bembrops*, and *Antigonia*.

iv — Deeper yet, near and below 400 m on the upper continental slope, the fauna changes to an assemblage (F) dominated by *Chaunax*, *Halosaurus*, *Epigonus*, *Galeus*, *Citta*, and *Trigla*.

v — Besides the species which most investigators agreed fell into one of the above assemblages, a few, such as *Cynoglossus canariensis* and *Brachydeuterus auritus*, were hard to place in any one assemblage because of their great bathymetric range and apparently wide ecological tolerance; they have been referred to as eurybathic species.

All the above assemblages were recognized on a subjective basis. When the data from the recently completed Guinean Trawling Survey became available, it seemed worthwhile to apply an objective grouping technique (Fager, 1957, 1963) to see whether or not the same assemblage would be obtained by its use and whether finer details of the structure of the communities would be revealed, and to explore more closely than had been previously possible the interrelationships of the assemblages with each other and with their environment. This paper reports the results of this and compares the objective with the subjective groupings: we believe that the results are of general interest for resource assessment studies in demersal fisheries, at any latitude, which are based on multispecies stocks.

MATERIALS

The data available for analysis were the species lists from the many stations worked with otter trawls during the Guinean Trawling Survey (GTS), organized in 1963–64 as Project 19 of the Commission for Technical Cooperation in Africa (CCTA) with its headquarters in Lagos, Nigeria. GTS was performed by two chartered trawlers very closely matched as to fishing power, that worked simultaneously over a pattern of trawl stations disposed along 63 transects spaced at 40-mile intervals the length of the tropical West African coast from Cape Roxo, south of Senegal, to the mouth of the Congo River (Fig. 1). Estuarine regions were not trawled.

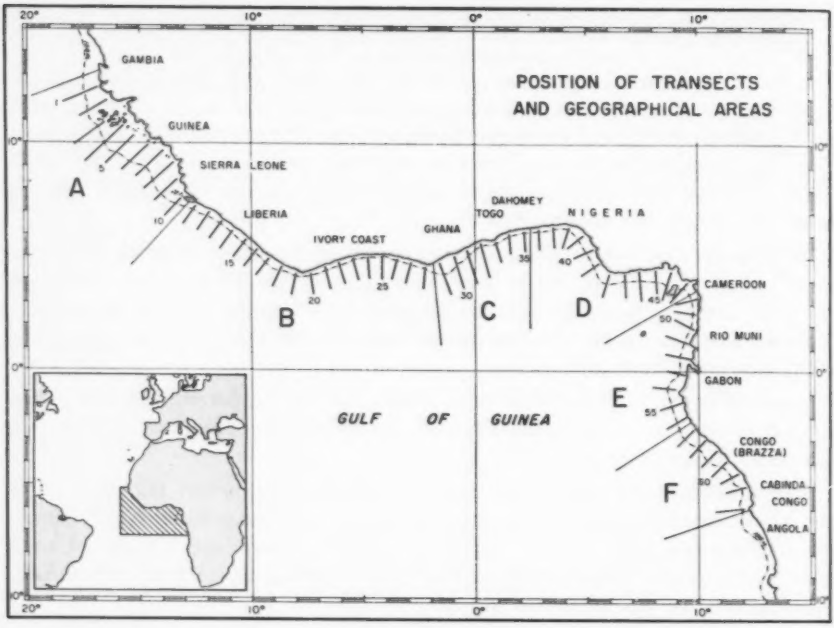

FIG. 1. The eastern tropical Atlantic Ocean, showing the location of the 63 transects of the Guinean Trawling Survey and the six subdivisions of this region used here for zoogeographical analysis.

On each transect eight stations were worked at the following series of standard depths: 15–20, 30, 40, 50, 70–75, 150, 250, and 400–600 m. Bathymetric control was very close during each haul and was checked by a bathykymograph mounted on the headrope of the trawl. Each station consisted of a simple suite of hydrographic and biological observations, followed by a 1-hr haul with standard otter trawls having headropes of 25 m and bridles of 37 m. Data derived from a total of 480 such stations were available for this analysis. Because of memory limitations of the computer, only the 175 most frequently occurring taxa in the lists were considered — these taxa were predominantly fish species, though some larger taxonomic units of fish and a single economically important species of penaeid prawn were also included.

GTS consisted of two surveys; Guinean I (GI) during September–December 1963, and Guinean II (GII) during February–May 1964. The first survey was during the transition from the wet to the dry season, soon after the peak effects of runoff from the rains of the preceding 3–4 months, and during a period of steadily rising insolation and mixed-layer temperatures. The second survey was at the height of the dry season, when temperatures would be at a maximum for the year and the influence of freshwater runoff minimal. The area of the survey was almost entirely within the tropical region

dominated by the Guinea Current and only marginally within the transitional zones between this current and the eastern boundary currents to the north and south. Within this tropical region, as the description of Berrit (1962) in particular shows, the hydrographic regime is remarkably constant, with the single exception of the localized area of seasonal coastal upwelling off Ghana and the Ivory Coast.

Records of the type of bottom deposit were based on the debris collected in the codend or adhering to the otter boards of the trawl at the end of the haul.

Hard-substrate environments were encountered mostly on inshore rocky reefs (off such localities as the Sierra Leone Peninsula), and on the offshore fossil coral banks that have been found along almost the entire coast near the break of slope on the continental shelf, generally at depths of 60–80 m (Allen and Wells, 1962). The rest of the West African continental shelf is covered by sandy or muddy deposits; the occurrence of mud depends in part on the presence of rivers, although the deposits outside the break in slope of the continental shelf are almost invariably muds. Because of the effluents of the Niger, Cross, Cameroon, and Congo rivers, bottom deposits at depths less than 80 m in the area east of Cotonou were predominantly muddy (90% of stations); to the west of Cotonou sand and rocks were much more frequent (43% of stations) at those depths.

METHODS

The computer program for grouping of taxa (REGROUP) was described by Fager (1957, 1963). It uses as an index of affinity the geometric mean of the proportion of joint occurrences, based on records of presence and absence, and is corrected for sample size. In the present study, the value of 0.5 was used as a breakpoint, only indices above this value being considering evidence of affinity. Thus, species whose frequencies of occurrence differed by more than a factor of four would not show affinity. The formation of recurrent groups was based primarily on two rules: firstly, that every taxon within a group must show affinity with all other members of the group, thus ensuring that every taxon in a group frequently co-occurs with every other member; secondly, that the largest possible groups must be formed. Species that showed affinity with some, but not all, of the members of a group were listed as associates of the group.

After the recurrent groups were formed, the connections between them were determined in terms of the number of species in each group that showed affinity with species in other groups (CONNEX). These are reported as fractions of possible connections, e.g., for two groups of three or four species each, there are 12 possible connections; an intergroup connection would be considered present and meaningful if at least three (0.25 of possible) intergroup pairs of species showed affinity. The distributions of occurrences of groups among the stations were also listed (STATION).

The recurrent groups were formed separately for GI and GII. In both surveys, over half of the taxa (91 and 97, respectively) did not occur consistently enough with others to show affinity at the level chosen. The taxa that did show affinity at this level (84 and 78) were either assigned to groups or noted as associates of these groups.

A comparison of the composition of the recurrent groups derived from the REGROUP program for GI and GII is made in Table II. Although only two groups (15 and 20) were identical in both surveys, most could be recognized in both on the basis of having at least some taxa in common, especially when associates were considered.

Most of the differences between the two surveys involved only changes in apportionment of taxa among groups shown to be related by CONNEX and STATION. Groups 1–3 provide a good example: in GI most of the taxa were put in group 1, whereas in GII they were distributed more evenly among all three groups. There were just two exceptions to this general rule: three groups (GI 13 and 17, GII 21) could not be matched between the surveys because the taxa included had significant affinity indices on only one survey; two taxa, *Sphyraena* spp. and *Lagocephalus laevigatus*, were put in group 19 in GII and were, respectively, a member and an associate of group 7 in GI. Because, as will be shown below, these two groups are members of two distinct multigroup assemblages, this difference appears to be evidence of a real seasonal shift in distribution. The other shifts between groups may also represent small seasonal changes in distribution or may be only the result of sampling variability. We cannot discriminate between these two possibilities on the basis of the present data.

Inspection of the lists of recurrent groups shows at once that they are considerably more numerous than are the subjective assemblages derived from previous work (Table I). To form larger assemblages that might correspond with the subjective communities, two ways of combining groups were tested, neither seeming at first sight to be of greater validity than the other: firstly, groups were combined on the basis of the species connections between them (CONNEX) when these reached a value of at least 0.25; secondly, combination was on the basis of frequency of joint occurrence of the groups (STATION) on individual transects, irrespective of the depth of haul. In this second method, groups were combined in an assemblage when they occurred jointly on transects more often than expected by chance (expectation based on total frequencies of occurrence; tested by chi square on 2×2 tables). The results of these two operations are summarized in Fig. 2 and 3, in which the assemblages have been arranged with the shallow-water groups near the top of the figure and the deepwater groups near the bottom.

In both surveys, only a few groups (GI 10, 13, 14, and GII 10, 12) were not marshalled into larger assemblages by connections based on affinities between component taxa. This method of forming assemblages tended to combine groups with similar depth ranges (cf. Fig. 2 and 3) and the groups show close agreement with the subjectively recognized communities listed in

TABLE II. Composition of the recurrent species groups (REGROUP), indicating differences between the two cruises. The numbers after the broken lines in the lists indicate the placing of a taxon in the other survey; e.g., the number 6 in group 1 of GI indicates that *Cynoglossus canariensis* was put in group 6 of GI.

Group no.	Guinean I	Guinean II
1	*Brachydeuterus auritus*	*Brachydeuterus auritus*
	Galeoides decadactylus	*Galeoides decadactylus*
	Vomer setapinnis	*Vomer setapinnis*
	Pseudotolithus senegalensis	*Pseudotolithus senegalensis*
	Ilisha africana	*Ilisha africana*
	Arius spp.	– – – – 3
	Pteroscion peli	– – – – 3
	Drepane africana	– – – – 2
	Pomadasys jubelini	– – – – 2
	– – – – 6	*Cynoglossus canariensis*
2	– – – – 1	*Deprane africana*
	– – – – 1	*Pomadasys jubelini*
3	*Pseudotolithus typus*	*Pseudotolithus typus*
	Dasyatis margarita	– – – –
	– – – – 1	*Arius* spp.
	– – – – 1	*Pteroscion peli*
	– – – – 4	*Pentanemus quinquarius*
4	*Pentanemus quinquarius*	– – – – 3
	Cynoglossus browni	– – – –
5	*Scoliodon terranovae*	*Scoliodon terranovae*
	Ephippion guttifer	– – – – 1 assoc.
	– – – – 6	*Penaeus duorarum*
	– – – – 7	*Paragaleus gruveli*
6	*Penaeus duorarum*	– – – – 5
	Cynoglossus canariensis	– – – – 1
	Trichiurus lepturus	– – – – 1 assoc.
7	*Paragaleus gruveli*	– – – – 5
	Sphyraena spp.	– – – – 19
8	*Dentex angolensis*	*Dentex angolensis*
	Dentex congoensis	*Dentex congoensis*
	Paracubiceps ledanoisei	– – – – 8 assoc.
	Lepidotrigla laevispinis	– – – –
	Squatina oculata	– – – – 9
	– – – – 8 assoc.	*Lepidotrigla cadmani*
	– – – – 8 assoc.	*Eucitharus linguatula*
9	*Pentheroscion mbizi*	*Pentheroscion mbizi*
	Brotula barbata	– – – – 9 assoc.
	– – – – 8	*Squatina oculata*
10	*Peristedion cataphractum*	*Peristedion cataphractum*
	Zenopsis conchifer	*Zenopsis conchifer*
	Antigonia capros	– – – – 10 assoc.
	Synagrops microlepis	– – – –
	– – – – 10 assoc.	*Bembrops heterurus*

(Continued)

TABLE II. Composition of the recurrent species groups (REGROUP), indicating differences between the two cruises. The numbers after the broken lines in the lists indicate the placing of a taxon in the other survey; e.g., the number 6 in group 1 of GI indicates that *Cynoglossus canariensis* was put in group 6 of GI. — (*Concluded*)

Group no.	Guinean I	Guinean II
11	*Chaunax pictus*	*Chaunax pictus*
	Epigonus telescopus	*Epigonus telescopus*
	Moridae	Moridae
	Macrouridae	Macrouridae
	Benthodesmis	– – – – 11 assoc.
	Galeus polli	– – – – 11 assoc.
	Setarches guentheri	– – – –
	Merluccius spp.	– – – –
	– – – – 11 assoc.	*Dibranchus atlanticus*
	– – – – 12	*Aristeus varidens*
12	*Cyttus roseus*	*Cuttys roseus*
	Aristeus varidens	– – – – 11
	Halosaurus oweni	– – – – 11 assoc.
	Etmopterus pusillus	– – – –
	– – – –	*Paracubiceps multisquamis*
13	*Trachinocephalus myops*	– – – – No similar
	Bothus podas africanus	– – – – group
	Xyrichthys novacula	– – – –
14	*Lutjanus fulgens*	– – – – No similar
	Bodianus iagonensis	– – – – group
15	*Lathrinus atlanticus*	*Lrtheinus atlanticus*
	Balistes forcipatus	*Balistes forcipatus*
16	*Acanthurus monroviae*	*Acanthurus monroviae*
	Lutjanus agennes	– – – – 16 assoc.
	– – – –	*Chaetodon luciae*
17	*Pagrus ehrenbergi*	– – – – 19
	Chilomycterurus spp.	– – – –
18	– – – – 19 assoc.	*Dactylopterus volitans*
	– – – –	*Balistes capriscus*
19	*Priacanthus arenatus*	*Priancathus arenatus*
	Fistularia villosa	*Fistularia villosa*
	Pseudupeneus prayensis	*Pseudupeneus prayensis*
	Pagellus coupei	*Pagellus coupei*
	Raja miraletus	*Raja miraletus*
	Ephinephelus aeneus	– – – – 19 assoc.
	– – – – 17	*Pagrus ehrenbergi*
	– – – – 7	*Sphyraena* spp.
	– – – – 7 assoc.	*Lagocephalus laevigatus*
20	*Boops boops*	*Boops boops*
	Scomber japonicus	*Scomber japonicus*
	Trachurus spp.	*Trachurus* spp.
21	– – – – No similar	*Hypoclydonia bella*
	– – – – group	*Chascanopsetta lugubris*

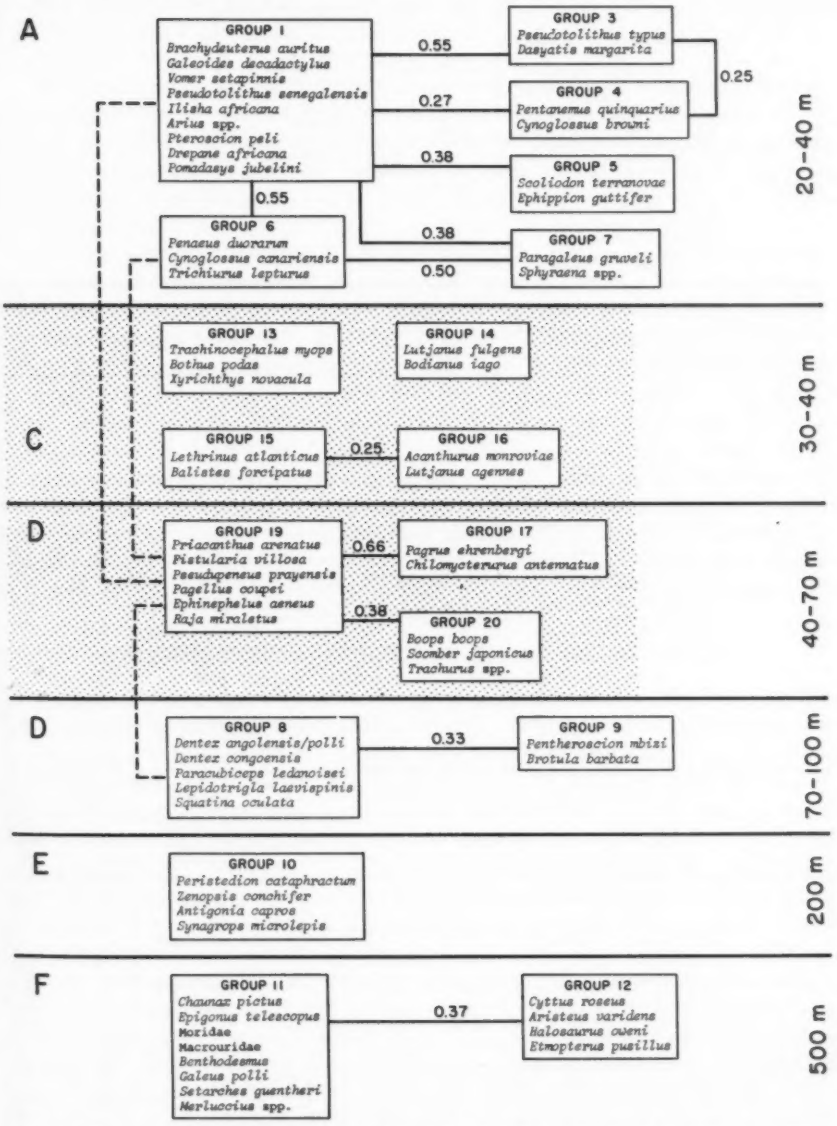

FIG. 2. The composition of species groups from GI and their marshalling into major assemblages or communities. Lines linking the groups indicate intergroup affinities; where the value from CONNEX equals or exceeds 0.25 it is indicated numerically on the lines that link the groups; broken lines indicates values less than 0.25. The stippled area of the figure indicates the second assemblage given by the analysis based on common occurrence on transects (STATION).

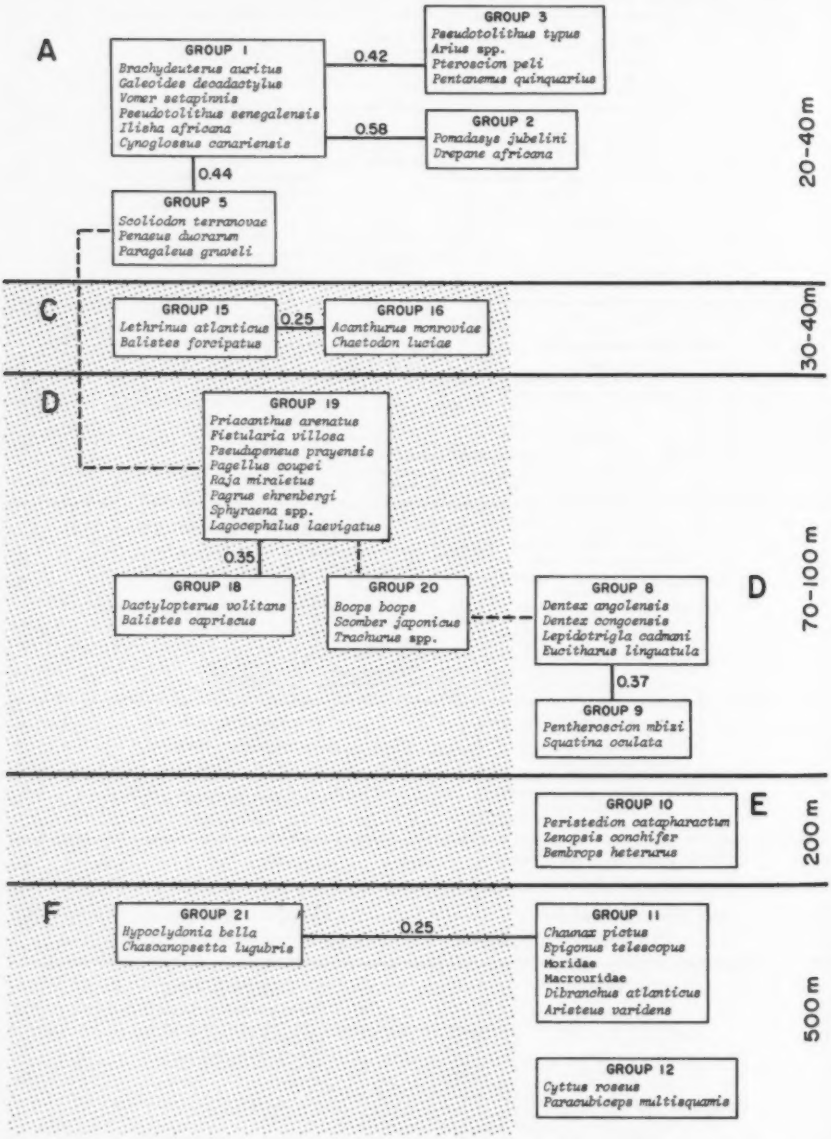

FIG. 3. The composition of species groups from GII and their marshalling into major assemblages or communities. Lines linking the groups indicate intergroup affinities; where the value from CONNEX equals or exceeds 0.25 it is indicated numerically on the lines that link the groups; broken lines indicate values less than 0.25. The stippled area of the figure indicates the second assemblage given by the analysis based on common occurrence on transects (STATION).

Table I. (The letters on the figures indicate the relationships; assemblage B does not occur on Fig. 2 and 3 because the surveys did not extend into estuarine environments.)

The analysis based on joint occurrences of groups on transects resulted in the formation of only two assemblages (in Fig. 2 and 3 and Table III). Groups placed in different assemblages occurred together on transects less often then expected by chance; consequently this second method of analysis tended to put together groups that occurred over similar substrates (cf. Table III, last column).

It is evident from Fig. 2 and 3 that although the two methods of combining groups gave different results, the major difference is one of scale, the method using intergroup affinities produced more, and smaller, assemblages. In only one case (groups 11 and 21 in GII) did one method suggest a connection where the other suggested a separation. Both methods separated the subjectively formed assemblage D into two components. Both methods gave remarkably similar results with the independent sets of data from the two cruises, GI and GII; most of the intercruise differences between the assemblages being similar in nature to the differences between groups on the two cruises. Because the two methods of forming assemblages emphasize different aspects of the environment, they give complementary results. Because the method using intergroup species affinities gives more detail, it may be the method of choice in future employments of this method of analyzing species assemblages.

COMPARISON OF OBJECTIVE AND SUBJECTIVE GROUPINGS

Most of the assemblages based on intergroup species affinities showed no connections, although assemblages A and D were exceptional in that a few species of one group in A (6 in GI, 5 in GII) had affinities at less than the 0.25 level with some species of group 19 in D. Several of these species in groups 5 and 6 (*Cynoglossus canariensis*, *Trichiurus lepturus*, *Scoliodon terranovae*, *Penaeus duorarum*, and *Paragaleus gruveli*) were previously considered as eurybathic and unassigned to a community. The results of the present analysis confirm that they have a wide depth range, but occur most frequently at depths between those favored by most of the groups in assemblage A and most of those in assemblage D.

An examination of the species groups forming assemblage A reveals only one group with any evident ecological significance: that which contains *Pseudotolithus typus* in each set of data. This sciaenid occurs more frequently in estuaries than on the open shelf, and it is significant that it occurs together with *Dasyatis margarita* in GI and with *Arius* spp., *Pteroscion peli*, and *Pentanemus quinquarius* in GII, for all of these are also normally common in estuarine samples. The two groups (GI 3 and GII 3), therefore, may represent the fauna which was taken on the shelf in both seasons when estuarine conditions were approached, and previously termed the estuarine sciaenid subcommunity.

TABLE III. The composition and ecological distribution of species groups. *No. stations* = number at which the whole group occurred together; *temperature range and depth distribution* = medians and ranges, as relevant; *geographic distribution*: A = transects 1–10 (Cape Roxo to Freetown), B = 11–28 (Freetown to Cape Three Points), C = 29–35 (Cape Three Points to Cotonou), D = 36–47 (Cotonou to Douala), E = 48–56 (Douala to Cape Lopez), and F = 57–63 (Cape Lopez to Congo River); *bottom deposit*: M = mud, SM = sandy-mud and muddy-sand, S = sand, SR = sand and gravel or rocks, R = rocks or coral. The broken line separates the two major assemblages based on joint occurrence on transects.

Group	Survey	No. taxa	No. stations	Temp range (*C*)	Depth (*m*)	Geographic distribution	Bottom deposit
1	I	9	30	19–27	20 (15–50)	D,F	
	II	6	45	20–45	30 (15–50)	D,F	M,SM
2	II	2	31	21–30	30 (15–40)		
3	I	2	30	19–28	20 (15–40)	D,F	
	II	4	16	20–29	20 (15–50)	D,F	
4	I	2	17	19–28	20 (15–50)	D,F	
5	I	2	71	18–29	30 (15–70)		SM
6	I	3	63	16–27	40 (15–70)	D	
7	I	2	72	17–27	40 (15–100)	D	
8	I	5	35	14–20	70 (70–200)		
	II	4	50	14–22	100 (40–200)	D,E	
9	I	2	54	14–22	100 (40–200)	D	M
	II	2	55	12–22	100 (50–200)	D	
10	I	4	15	13–16	200 (200–200)		
	II	3	17	12–17	200 (200–200)	E,F	
11	I	8	9	9–10	500 (400–600)		M
	II	6	8	8–10	500 (400–600)	E,F	M
12	I	4	7	9–10	500 (400–600)	D	M
	II	2	14	7–16	500 (200–600)	D,E	M
13	I	3	14	20–27	30 (15–50)	A	S
14	I	2	13	9– 4	40 (15–70)		SR,R
15	I	2	25	17–25	30 (15–50)		S,SR
	II	2	24	18–28	30 (15–40)	A,C	S,SR
16	I	2	13	17–24	40 (15–50)		SR,R
	II	2	13	18–25	40 (15–50)	C	SR
17	I	2	67	19–24	40 (15–70)		SM
18	II	2	44	18–28	40 (15–70)		S,SR
19	I	6	44	17–23	50 (15–70)		
	II	8	31	17–24	50 (15–70)		
20	I	3	45	15–23	70 (40–200)	A,C	SR
	II	3	36	16–21	70 (30–100)		
21	II	2	21	8–16	500 (200–600)		

It is also perhaps significant that the small sand shark *Scoliodon terranovae* occurs only in small species groups and shows no affinities (though it is a commonly occurring species) with any of those recurrent groups in either cruise which include the bulk of taxa typical of the offshore sciaenid subcommunity. This accords well with the frequently made observation that this

species and the normal teleosts of the community tend to be mutually excluding in the trawl catches, perhaps suggesting that when an aggregation of sand sharks moves into an area, the teleosts move out.

Groups 15 and 16 of GI and GII, together with their associated species, clearly from a community that is stable between the two cruises, and corresponds most closely with community C (Table I), the supra-thermocline sparid community of Longhurst (1963). There are, however, certain important differences. The absence of *Pagrus ehrenbergi* and *Albula vulpes* and the restriction of the group to reef-inhabiting species suggest that the shallow-water sparid community proposed by Longhurst was an artificial group; assemblage C as defined by REGROUP evidently represents the fauna occurring on the Holocene coral banks of the continental shelf, where there is known to be a fauna of lutjanids, lethrinids, and other reef fish that were included previously as part of the sparid community. The present analysis indicates that they have no affinity with any other group, and must form an assemblage of their own, to be designated the lutjanid community. The apparent occurrence of these species on a variety of deposit types is not to be taken too seriously, since they were probably caught when a trawl momentarily crossed over, or passed very close to a reef out-cropping from other deposits.

The groups comprising assemblage D require some investigation. In each cruise two groups (17 and 19 in GI, 18 and 19 in GII) occurred at depths and temperatures corresponding to the bottom of the thermocline, yet extended upwards into the mixed layer and temperatures above 25 C. These groups include at least one species (*Pagrus ehrenbergi*) previously included in the supra-thermocline sparid subcommunity, together with a number of ecologically similar forms (*Pagellus*, *Priacanthus*, *Dactylopterus*, and *Epinephalus*) and at least one (*Raja miraletus*) that was previously unallocated because it was considered a eurybathic species. In both cruises, these groups occurred rather frequently on hard deposits, and we now believe that these are the species which represent the element of the sparid complex that is able to penetrate above the thermocline onto sandy or broken corally deposits avoided by the sciaenid community. The evidence further suggests that even below the thermocline these species tend to occur together.

In the data from each cruise, there was a remarkably stable group (20) associated with the groups discussed above, composed of three species of semipelagic taxa: *Boops boops*, *Scomber japonicus*, and *Trachurus* spp. These are cold-water species which in the Canary and Benguela Current form surface shoals and which in the tropical Gulf of Guinea occur only in a rather narrow depth and temperature range and are most abundant near the bottom of the thermocline at about 17 C. The degree of association observed suggests that they form mixed shoals.

Groups 8 and 9 of both GI and GII extend deeper and occur more frequently on soft deposits than the other groups put in assemblage D and it is these which form the typical subthermocline sparid community, dominated by species of *Dantex*, *Lepidotrigla*, *Paracubiceps*, *Uranoscopus*, and *Pentheroscion mbizi*.

The deep shelf community (E) was identifiable as group 10 of both GI and GII, and the environmental properties showed that it was restricted to depths of around 200 m and to temperatures below 15 C. There was a rather large gap in sampling between 200 and 400 m on each transect and somewhere between these two depths the fauna changed completely to that represented by groups 11 and 12, and 11 and 21 of GI and GII, respectively. This continental slope community (F) occurred at temperatures between 7 and 10 C. In earlier studies it was recognized only by Salzen (1957). As might have been expected, this poorly known slope community was not consistently represented in the analyses of the data from the two surveys and was shown to include some ecologically based diversification. In Fig. 3 the GII data demonstrated that the species of group 21 *Hypoclydonia bella* and *Chascanopsetta lugubris*) were included in the assemblage of species-groups co-occurring on some transects where sandy and stony substrata dominated. Their co-occurrence with group 11 in some trawl hauls, however, suggests that group 21 may represent the fauna on patches of sandy deposit in regions of a mosaic of sandy and muddy substrata on the upper parts of the continental slope.

FACTORS INFLUENCING THE DISTRIBUTION OF GROUPS

The depth distribution of each group was examined (Table III) by coding the depths (0 = 15–20 m, 1 = 30 m, 2 = 40 m, . . .), calculating the mean coded depth of occurrence and then comparing the observed distribution of coded depths of occurrence for the groups with that expected for a Poisson distribution with this mean. For the shallow-water groups in both seasons (1–7, 13–18; both GI and GII), none of the observed distributions were significantly different from expectation (all $p \geq 0.10$, Kolmogorov–Smirnov goodness-of-fit test; Tate and Clelland, 1957). This finding indicates that these groups were not distributed evenly in the upper 70–100 m but that each had a favored depth, above and below which it occurred randomly; these groups showed the same sort of distribution of depth that one would expect from an underwater vehicle trying to maintain a certain depth but subject to random error in measurement of, or adjustment to, depth.

The information on the groups in deeper water is not strictly comparable because of the wider spacing of the deeper trawl hauls. With this limitation, the data suggest that some deeper groups were not randomly distributed with depth; the observed distributions generally were either sharply truncated upwards or limited to a single depth among those fished.

By and large, comparable groups in the two survey periods occupied similar depth ranges and, as temperature generally followed depth directly, experienced similar temperature regimes. The greater median depths observed in GII for a few groups (1, 2, 5, and 8) provide slight evidence that they, or at least some of their members, moved into slightly deeper water in the warmer season; these groups were all members either of the sciaenid community or of that element of the sparid community living on muddy ground below the

thermocline. No group typical of sandy or rocky substrates showed changes in its median depth range between the two cruises; this is probably an expression of the depth restriction of the hard sandy and stony deposits over most of the survey areas. It is also possible, of course, that this is related to the fact that the depths of occurrence of shallow-water groups, principally on sandy bottoms (groups 13–18; median depths, 30–40 m), were significantly greater ($p < 0.05$, Mann-Whitney U test; Tate and Clelland, 1957) than those for the groups typical of muddy bottoms (groups 1–7; median depths, 20–40 m). Perhaps those species that prefer shallow water over mud and sandy mud move inshore or offshore in response to seasonal changes in the environment, whereas those usually found over sand or rock do not undertake such movements because they live deeper where seasonal effects are less.

The frequencies of occurrence of five types of bottom deposit — mud, sandy mud and muddy sand, sand, sand and gravel or rocks, and rocks or coral — were tabulated for all stations. The distributions of observed frequencies of occurrences of the species groups at stations representing each major type of bottom deposit were compared with the distribution expected on the hypothesis of independent distribution of groups and bottom deposit types (K–S test; Tate and Clelland, 1957). There was a clear separation of the species groups into two assemblages corresponding to those based on joint occurrences of transects (Table III); one assemblage tended to occur more frequently than expected over mud and sandy mud substrates, and the other over sand and rocks. In their relation to substrate type, the groups in the second assemblage showed, proportionately, a greater number of biased distributions (at <0.10) than those in the first assemblage.

To consider geographic distribution of the species groups, the GTS area was subdivided; transects at which any group either began or ended a continuous distribution (present on three or more contiguous transects) were tabulated and those transects with the largest numbers of either terminations or starts were chosen to form six geographical subregions between Cape Roxo and the Congo River, with boundaries near Freetown, Cape Three Points, Cotonou, Douala, and Cape Lopez (Fig. 1, Table III). The frequencies of occurrences of the species groups in these six regions were compared with those expected on the hypothesis of equal probability of occurrence on all transects (K–S test; Tate and Clelland, 1957); this showed (Table III) that the groups and assemblages typical of muddy deposits were found more frequently than expected by chance in the three regions from Cotonou eastwards (D, E, F), and those typical of sandy substrata, though they did not as often show biased distributions (at $p < 0.10$), tended to be more frequent west of Cotonou (A, B, C).

This apparent difference in distribution was probably mostly related to the distribution of sediment types; for example, 83% of all stations with sand or rock bottom were in regions A–C. It cannot be attributed to the distribution of bottom types entirely, however, because only slightly over half (56%) of the stations with mud or muddy sand were in regions D–F. Furthermore

on the assumption that D, E, and F were equally likely to be occupied by groups preferring mud and A, B, and C by those preferring sand, significantly too many groups favored region D and too few favored region B.

CONCLUSIONS

The analysis presented and discussed here has confirmed in an objective manner the reality of the communities of demersal fish whose existence on the West African continental shelf had been previously postulated independently by a number of observers from their subjective treatment of trawling survey data. This agreement not only increases the reliance which may be placed upon this objective method of analysis of recurrent species groups, previously tested on zooplankton (Fager and McGowan, 1963; Sheard, 1965), but also confirms the likelihood that subjective judgments of the sort also being considered here may, in fact, be valid and useful; they should not be neglected in fishery exploration when more refined methods cannot be applied to the data.

One of us (Longhurst, 1967) has suggested that a search of the literature on trawling surveys in all parts of the tropical oceans has produced data which may subjectively be arranged to indicate that species assemblages analogous to those whose occurrence in the eastern Atlantic is here confirmed may also occur throughout the tropical Atlantic and Indo-Pacific, at least in environment similar to those in the Gulf of Guinea.

This computer analysis, by confirming the reality of these communities in one part of the tropical ocean, lends weight to the supposition that demersal fish communities on level sea bottoms on the tropical continental shelf, may generally, or at least frequently, be organized in a rather simple fashion similar to that described here.

ACKNOWLEDGMENTS

This work was supported in part by the National Science Foundation (Grant G-7141, GS-5800); in part by the Marine Life Research Program (Scripps Institution component of California Cooperative Oceanic Fisheries Investigation, sponsored by the Marine Research Committee of California); and in part by the Bureau of Commercial Fisheries of the U.S. Fish and Wildlife Service (contract 14-17-0007-139, 221 with Scripps Tuna Oceanography Research Program).

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North American Species of *Taeniopteryx* (Plecoptera, Insecta)[1]

By W. E. Ricker

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

AND

H. H. Ross

Illinois Natural History Survey
Urbana, Ill., U.S.A.

ABSTRACT

Keys, synonymy, descriptions, and distribution records are given for the eight American stonefly species of the genus *Taeniopteryx* Pictet, including the new species ***burksi***, ***lonicera***, ***metequi***, and ***ugola***.

INTRODUCTION

In the course of studying the distribution of the "winter" stoneflies of eastern North America it has been found that four undescribed species exist of the genus *Taeniopteryx* Pictet, and that the four described species have been variously confused in the past. This paper describes the new species, provides a key, and lists records of all species. Published records are assigned to their place in the present scheme insofar as the specimens have been available for re-checking.

Acknowledgment is made to the numerous individuals who have collected specimens, as indicated in the lists below; also to the various museums that have made their material available. Holotypes and allotypes of the species described here are in the collection of the Illinois Natural History Survey.

For the abundant species it has been impossible to list collections in detail except for a few that are of special interest. Otherwise a list is given of the counties where the species was taken, followed (in parentheses) by the number of sites where specimens were secured. In some cases collections were made at a particular site on more than one occasion, but this has not been indicated. In addition, the range of collection dates is indicated for each state or province as a whole (Ontario is divided into a southern and a northern section, corresponding to the political distinction between counties and districts).

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Complete details of the collections are available from the American Documentation Institute's Auxiliary Publication Service, c/o Library of Congress, Washington, D.C. The accession no. may be obtained from the authors.

KEY TO AMERICAN *TAENIOPTERYX*

MALES

1*a* Vesicle (ventral lobe) present on the 9th sternite (occasionally obsolescent or very rarely lacking in *burksi*, whose straight pointed subanal lobes will distinguish it from the species under 5) 2

1*b* Vesicle absent 5

2*a* Distal part of subanal lobes narrow, hooked at the tip; rare Appalachian species..... *ugola*

2*b* Distal part of subanal lobes broader, tapered to a straight subacute tip; abundant species.. 3

3*a* Hairs at the hind margin of the 9th sternite pointing downward and forward, usually much shorter than those situated more anteriorly on the sternite; vesicle 2–3 times as long as wide; aedeagus lacking a brown sclerotized band between the two lateral lobes (sometimes with an indistinct grey band); no trace of spurs or calluses on hind femora ..... *nivalis*

3*b* Hairs at the hind margin of the 9th sternite pointing mainly backward, much longer than the more anterior ones; vesicle 3–5 times as long as wide; extruded aedeagus with a strong band of brown sclerotization situated posteriorly between the two lateral lobes; femora with or without spurs 4

4*a* Hind femora with a spur at mid-length; its length equal to half to the whole of the width of the femur ..... *maura*

4*b* Hind femora either unmodified or with some thickening of the hind margin at mid-length (this occasionally bears a sharp point, but is less than half as long as the width of the femur) ..... *burksi*

5*a* Distal part of subanal lobes broad and flat, with a broadly rounded or subtruncate tip; cerci modified 6

5*b* Distal part of the subanal lobes narrow, and hooked or club shaped at the tip; cerci globular..7

6*a* Cerci (dorsal view) longer than the breadth of their base, appearing twisted; posteroventral surface of 9th sternite only slightly connate, not membranous though sometimes lighter in colour than the rest of the sternite ..... *lonicera*

6*b* Cerci no longer than their base, chunky; posteroventral surface of the 9th sternite elevated' pale, membranous, bearing long light twisty hairs ..... *lita*

7*a* Tips of subanal lobes hooked, i.e. subacute and bent outward ..... *parvula*

7*b* Distal part of subanal lobes (ventral view) straight, somewhat swollen, narrowly rounded at the tip ..... *metequi*

FEMALES

1*a* Notch of the 8th sternite deep, margined by a broadly U-shaped band of heavy sclerotization ..... *lita* and *lonicera*

1*b* Notch margined by only normal sclerotization, or a by narrow V-shaped dark marginal band 2

2*a* Middle half of the 8th sternite little sclerotized, light in colour, contrasting strongly with the central plate and narrow dark margin of the notch ..... *parvula*

2*b* Middle part of the 8th normally sclerotized, except sometimes for a light anterior spot near the mid-line 3

3*a* Sides of the notch, if produced inward and forward, meet at an obtuse angle.. *burksi* and *maura*

3*b* Produced sides of the notch meet at an acute angle; usually with strongly sclerotized "shoulders" near the hind margin of the sternite ..... *nivalis* and *metequi*

3*c* Produced sides of the notch meet at a right angle; shoulders less prominent ..... *ugola*

Taeniopteryx **burksi**, n.sp.

1925. *Taeniopteryx nivalis* Needham and Claassen (in part — not Fitch 1847).
1929. *Taeniopteryx nivalis* Frison.
1935. *Taeniopteryx nivalis* Frison.
1942. *Taeniopteryx maura* Frison (in part — not Pictet, 1841).
1945. *Taeniopteryx maura* Ricker (in part — all the Indiana specimens listed are *burksi*, except a few of those from Warsaw).

Adults and nymphs of this species have been described and illustrated by Dr Frison (1929, 1935) under the name *Taeniopteryx nivalis* (Fitch).

MALE: Similar to *T. maura* but usually averaging darker in colour and somewhat smaller in size (when taken in the same locality). The femora usually lack spurs completely, but sometimes have a low callus, rarely tipped by a definite angle or a small spine not more than half as long as the width of the femur. Terminal hairs of the 9th sternite longer than the more anterior hairs, directed mainly backward. Ventral lobe of the 9th sternite 3–5 times as long as wide, hence longer than in *nivalis*.

FEMALE: Not yet distinguishable morphologically from *maura*. The 8th sternite is shown in Fig. 11. The anterior pale spot is not always present. The two membranous appendages behind the central plate occur also in all other species of *Taeniopteryx*, Eurasian as well as American, but are sometimes retracted and illustrations often omit them (e.g., Fig. 7, 10). Frison's figure (1929, fig. 78; 1935, fig. 216) shows the central sclerotized plate to be at the hind margin of the sternite, although his text correctly states that it is "in reality merely in the posterior more membranous or light-coloured portion."

Where *burksi* and *maura* occur in the same locality, no overlap in spur size has been observed. In the Potomac River collections, for example, there is a wide gap in spur size between the two. Where only one species occurs, on the other hand, there may be less difference. For example, a few individuals in some of the New Brunswick *maura* populations had quite small spurs or, in one specimen, only calluses. Similarly, the collections of *burksi* from Elizabethtown and Oakwood, Illinois, had a rather high incidence of calluses and some small spurs, plus occasional individuals whose spurs were in the lower part of the *maura* range of sizes. Frison (1929, Fig. 64, 65) has illustrated femora with and without the callus, both probably from the Oakwood population.

The name *burksi* is for Dr B. D. Burks of the United States National Museum, whose collections were primarily responsible for distinguishing this species from *maura*.

Holotype ♂, allotype ♀, 8 ♂ and 11 ♀ paratypes, Urbana, Champaign Co., Illinois. T. H. Frison. Jan. 14, 1928. Other paratypes include the males among the collections below.

ALABAMA (December 29–January 26): Blount (1), Cherokee (1), Shelby (1).
ARKANSAS (January 4–March 21): Benton (2), Carroll (1), Franklin (1), Madison (4), Marion (3), Sharp (1), Washington (3), Yell (1).
DISTRICT OF COLUMBIA (January 23–March 20): (1).
GEORGIA (December 31–January 30): Dade (1), Fannin (2), Gilmer (1), Lumpkin (1), Towns (1), Union (2).

ILLINOIS (January 11–April 10): Champaign (4), Cumberland (1), Hardin (1), La Salle (2), Lawrence (1), Marshall (1), Rock Island (1), Tazewell (1), Vermillion (3).

INDIANA (January 25–April 5): Bartholomew (3), Carroll (2), Cass (1), Dubois (1), Fountain (2), Grant (2), Greene (1), Hamilton (1), Huntington (2), Jackson (1), Jasper (1), Jennings (1), Johnson (1), Knox (1), Kosciusko (2), Lawrence (1), Monroe (1), Montgomery (2), Morgan (1), Newton (2), Orange (1), Owen (1), Pike (1), Rush (1), Tippecanoe (4), Wabash (1), Warren (1), Washington (1), White (1).

IOWA (March 25): Jones (1).

KANSAS (March 15): Riley (1).

KENTUCKY (December 26–March 20): Allen (1), Carter (2), Clinton (1), Hart (1), Perry (1), Jackson (1), Rockcastle (1), Wayne (1), Whitley (2),

MAINE (March 23–"April"): Androscoggin (1), Cumberland (1), Lincoln (1), Penobscot (1).

MARYLAND (February 13–25): Allegany (1), Garrett (1), Village of Priest's Bridge (1).

MICHIGAN (March 10–April 3): Clinton (1), Ingham (3).

MISSOURI (January 2–March 24): Crawford (1), Dallas (1), Douglas (1), Franklin (1), Hickory (2), Laclede (1), Madison (1), St. Clair (1), St. Louis (1).

NEW BRUNSWICK (April 13–15): Queens (2), Westmorland (5).

NEW HAMPSHIRE (March 16–April 3): Belknap (1), Hillsboro (3), Merrimack (3).

NEW YORK (March 12–April 11): Broome (1), Hamilton (1), Herkimer (1), Jefferson–Lewis (1), Oswego (1), Niagara (1), St. Lawrence (4), Steuben (1), Sullivan (1), Tompkins (1).

NORTH CAROLINA (December 22–March 16): Durham (3), Haywood (1), Jackson (3), Johnstone (2), Macon (3), Swain (1), Wake (7), Yancey (1).

NOVA SCOTIA (April 15–20): Antigonish (1), Colchester (3), Cumberland (1), Guysboro (3), Halifax (4), Hants (1), Lunenburg (1),Pictou (1).

OHIO (February 5–March 19): Hocking (1), Preble (1), Scioto (3), Shelby (1).

OKLAHOMA (February 10–11): Latimer (1), Leflore (2).

ONTARIO — *north* (March 16–May 2): Algoma (2), Muskoka (1), Nipissing (4), Thunder Bay (6).

ONTARIO — *south* (March 15–April 10): Hastings (1), Renfrew (1), Waterloo (1), Wellington (1).

PENNSYLVANIA (February 13–March 23): Cambria (1), Cameron (1), Dauphin (2), Lycoming (2), Sullivan (1), Union (1).

QUEBEC (March 30–April 19): Gatineau (4), Labelle (3), Laviolette (1), Maskinongé (1), Montcalm (1), Papineau (1), Portneuf (3), Québec (1), Temiskaming (3), Terrebonne (1).

RHODE ISLAND (March 3–7): Washington (1).

TENNESSEE (December 29–March 24): Blount (1), Coffee (1), Cumberland (2), Harding (1), Houston (2), Humphreys (3), Maury (1), Montgomery (3), Morgan (2), Sevier (3), Stewart (1), Sumner (1), Unicoi (1), Warren (1).

VIRGINIA (December 31–March 21): Fauquier (1), Giles (1), Madison (2), Montgomery (1), Prince William (1), Roanoke (2), Rockingham (2), Spotsylvania (1), Warren (3).

WEST VIRGINIA (January 25–March 18): Cabell (1), Clay (1), Hampshire (3), Hardy (1), Nicholas-Fayette (1), Kanawha (1).

WISCONSIN (March 23–April 10): Barron (1), Dunn (1), Shawano (1), Trempealeau (3), Vilas (2).

Taeniopteryx lita FRISON 1942

1929. *Taeniopteryx parvula* Frison (in part, not Banks, 1918). The specimens from Elizabethtown and Grayville are *lita*.

1935. *Taeniopteryx parvula* Frison (in part). The specimens from Elizabethtown, Golconda, and Grayville are *lita*.

1942. *Taeniopteryx lita* Frison (in part). Description and records from Illinois (type locality), Arkansas, and Indiana. The paratypes from Maryland and Mississippi are of the species *lonicera*.

Characters distinguishing the males of *lita* from the similar species *lonicera* are summarized on page 1428. The holotype is from the Ohio River at Elizabeth-

town, Illinois. The male specimens listed below have been re-examined and found to agree with the *lita* holotype; the females listed were either taken with males or are very probably *lita* because of their location and large size. At the margin of its range, *lita* has twice been taken in the same collection as *lonicera*, namely the Alabama and North Carolina records below.

ALABAMA: Route 69, 10 miles NE of Jasper, Walker Co., Ross and Ross; Jan. 12, 1963, 1 ♂
ARKANSAS: Arkansas R., Franklin Co.; L. O. Warren; Feb. 17, 1962, 1 ♀. Mill Cr., Polk Co.; L. O. Warren; Feb. 17, 1962, 1 ♀. Cache R., Brasfield, Prairie Co.; Ross and Ross; April 16, 1939, 1 ♂ (paratype). Petit Jean Cr., Hwy 154, Yell Co. at Conway Co. line; Hite and Wall; March 9, 1963, 2 ♀.
ILLINOIS: Ohio R. at Elizabethtown, Hardin Co.; Frison and Ross; March 7, 1928, 4 ♂ (holotype and paratypes), 5 ♀ (including allotype). Golconda, Pope Co.; H. H. Ross; March 17, 1932, 1 ♂ (paratype). Kaskaskia R. at Evansville, Randolph Co.; Frison and Mohr; Feb. 3, 1934, 1 ♀. Wabash R., Grayville, White Co.; Frison and Ross; March 8, 1928, 4 ♂ (paratypes), 14 ♀.
INDIANA: St. Anthony, Dubois Co.; Frison and Mohr; Feb. 13, 1938, 1 ♂ (paratype). Shoals, Martin Co.; 2 ♀. Patoka R., Winslow, Pike Co.; Frison and Mohr; Feb. 14, 1938, 2 ♂ (paratypes). White R. at Rogers, Pike Co.; Frison and Ross; March 14, 1936, 4 ♂ (paratypes), 1 ♀; March 19, 1936, 2 ♀.
KENTUCKY: Route 54 at Ohio-Grayson Co. line; Rotramel and Unzicker; Feb. 28, 1966, 1 ♂. Nolin R. at Millerstown, Hart–Grayson Co. line; Rotramel and Unzicker; March 3, 1966, 1 ♂.
MISSISSIPPI: Creek 2.5 miles SE of Iuka, Tishomongo Co.; Hensley and Smith; Jan. 8, 1965, 1 ♂.
NORTH CAROLINA: Neuse R. at Hwy 301, 5 miles S of Smithfield, Johnston Co.; Wray and Greene; Jan. 13, 1965, 4 ♂, 2 ♀.
TENNESSEE: Hatchie R., 4 miles S of Brownsville, Hayward Co.; Ross and Stannard; Feb. 21, 1963, 2 ♂, 3 ♀. Obion R., Obion, Obion Co.; Ross and Stannard; Feb. 20, 1963, 1 ♂. 1.2 miles W of Kempville, Smith Co.; Rotramel and Unzicker; March 2, 1966, 1 ♂. Dillard Cr., 4 miles NE of Chestnut Mound, Smith Co.; Rotramel and Unzicker; March 2, 1966, 1 ♂, 5 ♀.

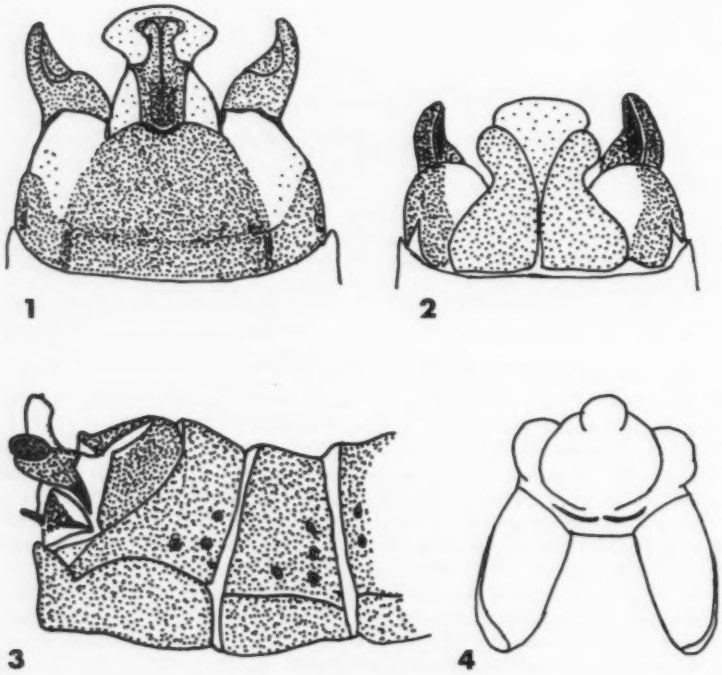
Taeniopteryx ***lonicera*** n.sp.

MALE: Length of body 5.5–7 mm, to tip of wings 9–10 mm. Very similar to *T. lita*, but averages somewhat smaller. 9th sternite without a vesicle, bluntly connate at its posteroventral angle (Fig. 3), and usually bears a white spot behind this angle near the hind margin. 10th tergite bent downward for the posterior 0.7 of its length, the hind margin sclerotized and excavated (Fig. 1, 3). Epiproct, in side view, curved upward and forward; in front view angularly indented on the sides and smoothly rounded at the tip, with a median sclerotized pattern as in Fig. 1. Subanal lobes (ventral view) divergent at the tips, smoothly rounded (Fig. 2). Cerci sclerotized and twisted, longer than their basal width (top view). Extruded aedeagus (rear view) with two long downward-pointing lobes grooved along the outside, and 2 short dorsolateral lobes, the former joined by a narrow line of sclerotization (Fig. 4).

FEMALE: Length of body 7.5–8.5 mm, to tip of wings 10–12 mm. Not yet distinguishable from *T. lita*, which is illustrated by Frison (1942, p. 250).

The points of difference between the males of *T. lonicera* and *T. lita* are summarized below. Points 1 and 2 are invariably diagnostic; 3 and 4 are less easily described and in some respects tend to intergrade. For females the smaller size of *lonicera* often permits a good guess as to which species is involved.

	lonicera	*lita*
Cerci	Inner surface deeply excavated on the distal 0.7 of its length; appearing slender and twisted	Inner surface shallowly excavated on the distal 0.4 of its length; appearing short and massive
Ninth sternite	Posteroventral median projection a low flattened cone, not unusually hairy; often with a small light spot behind it	Posteroventral projection swollen, light in colour and with a depression at the tip, and bearing long irregular whitish hairs
Subanal lobes	Distal portion bent outward, evenly rounded	Distal portion usually nearly straight on the inner margin but expanded on the outer side; hind margin often nearly straight
Epiproct	Deeply excavated on the sides; tip rounded; median pattern much expanded near the tip	Excavated only shallowly on the side; tip flatter, sometimes straight; median pattern less expanded near the tip



Figures 1–4. *Taeniopteryx lonicera.*

FIG. 1. Male 10th tergite and appendages.

FIG. 2. Male 10th segment from below (9th sternite removed).

FIG. 3. Male terminalia, from the side.

FIG. 4. Extruded aedeagus, from behind.

The range of *T. lonicera* embraces much of the region whose roadsides are made fragrant in summer by vine honeysuckle. It is abundant from North Carolina to Alabama and overlaps the range of *lita* into Tennessee.

Holotype ♂, allotype ♀, 7 ♂ and 13 ♀ paratypes: Laurel Fork, S of Blountsville, Blount Co., Alabama, H. H. Ross and J. A. Ross, Jan. 26, 1958. Other paratypes as follows (males only):

ALABAMA (except where noted, all collections were made by H. B. Cunningham): Persimmon Cr., 4 miles S of Georgiana on Route 31, Butler Co.; Dec. 8, 1967, 3♂, 1♀. Choccolocco Cr., Calhoun Co.; Dec. 14, 1967, 1 ♂, 4♀. Abanda, on Ala. Route 77, Chambers Co.; Dec. 21, 1967, 2 ♂, 1 ♀. Large creek 3 miles N of Galesville, Cherokee Co.; Dec. 31, 1963, 5 ♂, 12 ♀. Ellisville, Cherokee Co.; Dec. 13, 1967, 1 ♂, Panther Cr. on Route 31, Castleberry, Conecuh Co.; R. E. Waters, Feb. 1, 1966, 2 ♂; H. B. Cunningham, Dec. 8, 1967, 1 ♂. Junction Murder Cr. and Route 31, S of Evergreen, Conecuh Co.; Dec. 8, 1967, 1 ♂. Burnt Corn Cr., Brewton, Escambia Co.; Dec. 9, 1967, 1 ♂. 0.5 S of mile Attalla, Etowah Co.; Jan. 4, 1964, 2 ♂, 3 ♀. S of Swaim on Ala. Route 65, Jackson Co.; Dec. 14, 1967, 2 ♂, 1 ♀. 2 miles S of Garth on Ala. Route 65, Jackson Co.; Dec. 14, 1967, 1 ♂, 1 ♀. 1 mile S of Hollytree on Route 65, Jackson Co.; Dec. 14, 1965, 2 ♂, 1 ♀. 1.4 miles S of Junction Roads 48 and 43, on 48, Lee Co.; Dec. 2, 1967, 1 ♂, 1 ♀. 2.5 miles SE of Junction of Routes 12 and 169, on 169, Lee Co.; Dec. 2, 1967, 1 ♂. 2 miles W of County line (Madison) on U.S. 12, Limestone Co.; Dec. 14, 1967, 2 ♂, 2 ♀. S of Wattsville, St. Clair Co.; Ross and Ross. Jan. 25, 1958, 5 ♀. W edge of Asheville, St. Clair Co.; Jan. 4, 1964, 13 ♂, 7 ♀. E edge of Camp Hill, Tallapoosa Co.; Dec. 17, 1967, 1 ♂, 1 ♀. 1 mile W of Camp Hill on U.S. 280, Tallapoosa Co.; Dec. 17, 1967, 1 ♂. Sipsey Cr. on Ala. Route 60, Winston Co.; Dec. 15, 1967, 1 ♂.

GEORGIA: Oconee R., S of Athens, Clarke Co.; Hensley and Smith; Dec. 30, 1964, 1 ♂. 0.5 mile S of Armachee, Floyd Co.; Cunningham and Cunningham; Jan. 3, 1964, 18 ♂, 28 ♀. S edge of Calhoun, Gordon Co.; Cunningham and Cunningham; Jan. 3, 1964, 2 ♂, 1 ♀. 6 miles W of Fair Mount, Gordon Co.; Cunningham and Cunningham; Jan. 5, 1964, 19 ♂, 4 ♀. Fast Creek 1 mile NW of Talmo, Jackson Co.; Hensley and Smith; Dec. 30, 1964, 1 ♂, 1 ♀.

MARYLAND: Priests Bridge; B. D. Burks; Feb. 25, 1938, 1 ♂ (paratype of *lita* Frison).

MISSISSIPPI: Potts Camp, Marshall Co.; T. H. Frison; Feb. 17, 1941, 1 ♂ (paratype of *lita* Frison).

NORTH CAROLINA: Branch of Muddy Cr., Forsyth Co.; J. F. Greene; Jan. 8, 1963, 1 ♂. Buffalo Cr. at Hwy 42, 10 miles E of Clayton, Johnston Co.; Wray and Greene; Jan. 13, 1965, 1 ♂, 1 ♀. Little R., 13 miles E of Clayton, Johnston Co.; Wray and Greene; Jan. 13, 1965, 1 ♂, 1 ♀. Little R., 3 miles E of Kenly on Hwy 301, Johnston Co.; Wray and Greene; Jan. 13, 1965, 2 ♂, 12 ♀. Little R., 5 miles W of Kenly, Johnston Co.; Wray and Greene; Jan. 13, 1965, 6 ♂, 2 ♀. Neuse R. on Hwy 301, 5 miles S of Smithfield, Johnston Co.; Wray and Greene; Jan. 13, 1965, 4 ♂, 2 ♀. Little R., Voss, Moore Co.; R. D. Cuyler; Jan. 30, 1964, 2 ♂, 7 ♀. Bridge on Ralesville Road, Wake Co.; D. L. Wray; March 6, 1958, 1 ♂, 1 ♀. Neuse R., Wake Co.; Wray and Greene; March 6, 1958, 4 ♂, 9 ♀. Tar R., Rocky Mount, Nash Co.; Lee Griffin; March 2, 1968, 2 ♂, 1 ♀.

TENNESSEE (all collections were made by H. B. Cunningham): 4 miles NE of Whitwell, Marion Co.; March 13, 1964, 1 ♂, 2 ♀. 3 miles S of Dunlap, Sequatchie Co.; March 12, 1964, 1 ♂. 7 miles W of Powells Crossroads; March 13, 1964, 2 ♂, 1 ♀.

Taeniopteryx maura (PICTET 1841)

1841. *Nemoura maura* Pictet.

1925. *Taeniopteryx maura* Needham and Claassen.

1942. *Taeniopteryx maura* Frison (in part). In 1942 Frison used this same name to include *maura*, *burksi*, and *nivalis* of this paper.

Pictet's types of *maura* are lost; they were from "Pennsylvanie," where several species of *Taeniopteryx* occur. We follow the usage of Needham and Claassen (1925), who associated specimens having strong femoral spurs with this name. Their description and illustrations are all applicable to the name *maura* as used here, except that the illustration of the female 8th sternite (Plate 44, fig. 7) is not very distinctive.

MALE: Hairs at the hind margin of the 9th sternite longer than those farther forward on the sternite. Vesicle 3–5 times as long as wide. Aedeagus sclerotized heavily at the inside angles of the lateral bulbs and along a continuous line joining these across the central bulb (posterior view when extruded). Hind femora with a conspicuous spur near the middle, its length commonly almost or quite equal to the width of the femur, rarely as short as half this width.

FEMALE: Sides of the median notch of the 8th sternite forming an obtuse angle medially (if produced forward); curving gradually to the sides of the segment, without distinct "shoulders."

We have been unable to find consistent characters to distinguish females of this species from *burksi*. East of the Appalachians *T. maura* tends to be lighter in colour and larger in size than *T. burksi*, but west of the mountains this is not apparent. When the two species occur in the same locality, *maura* tends to emerge later in the season than *burski*; and its does not range as far north.

The peculiar femoral spurs of this species are matched by similar but more slender structures in two European species, *T. kühtreiberi* Aubert and *T. auberti* Kis and Sowa, but these two are not otherwise close to *maura* in any respect.

Taeniopteryx maura has a considerably more restricted range than *T. burksi*. It has been taken in Cape Breton Island and is fairly common in eastern New Brunswick, but there are as yet no records from the four northeastern States. It reappears in Connecticut, New York, and Pennsylvania, west to the northeastern corner of Ohio, but then retreats south of the Ohio River except for a single Indiana record. Southward *maura* is common down to Georgia and Alabama, but there is only one record for Arkansas.

ALABAMA (December 19–January 26): Blount (1), Calhoun (1), Chambers (1), Cherokee (1), Chilton (1), Cullman (2), DeKalb (2), Etowah (2), Fayette (2), Marion (5), Winston (3).
ARKANSAS: S Fork of Saline R. at Hwy 5, Garland Co. Hite and Aggus. March 17, 1963, 1 ♂.
CONNECTICUT (March 24): Fairfield (2), New Haven (1).
DISTRICT OF COLUMBIA (February 19–March 20): (2).
GEORGIA (January 3–30): Catoosa (2), Floyd (2), Gordon (1), Rabun (1), Towns (2), Union-Towns (1), Union (1).
INDIANA: Richland Cr. on Hwy 231, 1 mile S of Bloomfield, Greene Co. R. C. Dobson. February 4, 1960, 3 ♂.
KENTUCKY (December 26–March 14): Allen (2), Barren (1), Breckenridge-Grayson (1), Franklin (1), Green (2), Hart–Grayson (1), Jackson (1), Larue (1), Rockcastle–Laurel (1), Monroe (2), Ohio–Grayson (1), Oldham (1), Todd (1), Warren (2), Wayne (1).
MARYLAND (February 12–March 12): Allegany (1), Charles (1), Montgomery (1), Village of Priest's Bridge (1).
MISSISSIPPI: Potts Camp, Marshall Co. T. H. Frison. February 17, 1941, 2 ♂, 2 ♀.
NEW BRUNSWICK (April 14–15): Northumberland (1), Queens (1), Westmorland (2), York (3).
NEW YORK (March 3–April 8): Broome (1), Chenango (2), Delaware (2), Essex (1), Hamilton (5), Herkimer (1), Livingston (1), Ontario (1), Orange (1), Otsego (5), St. Lawrence (1), Schuyler (1), Steuben (2), Sullivan (3), Tompkins (1), Ulster (1), Warren (1).

NORTH CAROLINA (December 23–March 2): Alamance (1), Cherokee (1), Forsyth–Rockingham (1), Franklin (1), Haywood (1), Jackson (3), Johnston (6), Macon (3), Nash (1), Orange (2), Person (1), Randolph (1), Wake (6).

NOVA SCOTIA (April 18): Inverness (3).

OHIO: Creek E of Warren, Trumbull Co. W. E. Ricker. March 13, 1964, 1 ♂, 1 ♀.

PENNSYLVANIA (February 17–March 15): Cameron (1), Dauphin (1), Lycoming (3), Mercer (1), Potter (1), Sullivan (1), Tioga (1), Venango (1).

TENNESSEE (December 29–March 16): Coffee (1), Hickman (1), Humphreys (1), Knox (1), Marion (1), Maury (1), Montgomery (6), Robertson (1), Robertson–Montgomery (2), Sevier (6), Sumner (1), Wilson (1).

VIRGINIA (January 1–March 31): Allegheny (1), Giles (5), Montgomery (4), Rappahannock (1), Roanoke (2), Rockingham (1), Scott (1), Tazewell (1).

WEST VIRGINIA (January 25–March 17): Clay (2), Grant (1), Hampshire (1), Mineral (1), Nicholas–Fayette (1), Pocahontas (2).

Taeniopteryx ***metequi*** n.sp.

MALE: Length of body 6–7.5 mm, to tip of wings 5.5–7 mm. Wings usually a little shorter than the body, lightly infuscated, with slightly darker irregular bands at the cord and near the tip. Coastal crossveins typically include the humeral only, if Sc is considered to end in C; sometimes an apical crossvein is present; often the venation is somewhat irregular in various parts of the wing. Epiproct similar to *nivalis*. Cerci with 1 segment, only slightly modified from a globular shape (Fig. 6). Ninth sternite lacking a vesicle. Subanal lobes, viewed from behind, with an inner subacute terminal angle, and with a slender club-shaped process a little laterad of this, which may turn outward at the tip but usually does not (Fig. 5). Aedeagus (when extruded) shows no definite sclerotization, but there is sometimes a band of light grey in the dorsal angle of each lateral lobe and joining these across the posterior lobe; lateral lobes with a shallow to deep lateral groove, making them appear double; central lobe often with an acute nipple posterodorsally.

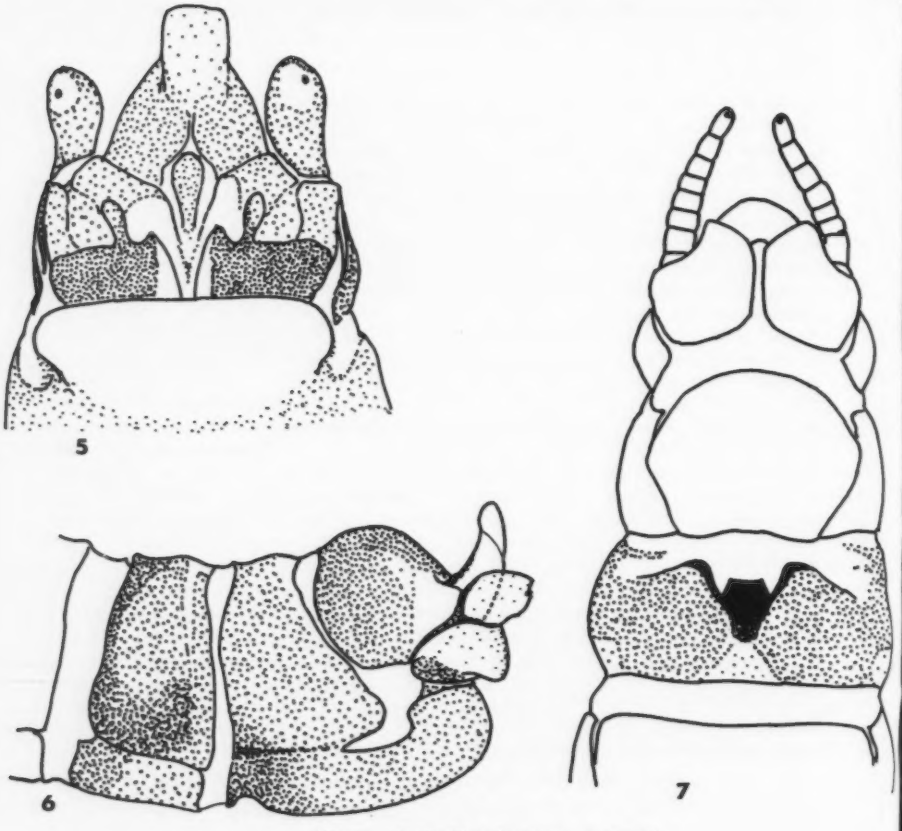
FEMALE: Length of body 8.5–10 mm, to tip of wings 9–11.5 mm. Wings somewhat shortened, usually a little longer than the body. Eighth sternite normally sclerotized anterior to the V-notch, except for a light patch in front of the median plate, and one at either side (Fig. 7). Median plate rather broad and heavy, the margins of the V-notch strongly sclerotized, terminating in a rounded shoulder that bends to the side and a little forward.

The male subanal lobes of this species are usually easily distinguishable from all others, but the club-shaped process is occasionally pigmented in a manner that suggests *parvula* on casual inspection, and several specimens were in fact confused with *parvula* in the past. However the mesal subacute process is not found in *parvula*. The female is very different from *parvula* and most resembles *nivalis*, but is smaller than the latter and the shoulder laterad of the V-line is typically bent a bit forward instead continuing sideways and a little back.

The distribution of this species coincides fairly well with that of the great broad-leaf hardwood forest of eastern North America — called metéqui in the Algonkian languages of Illinois.

Holotype ♂, allotype ♀, 16 ♂ and 10 ♀ paratypes. Hayes Creek, Glendale, Illinois, March 25, 1958, H. H. Ross and L. J. Stannard. Other paratypes as follows.

ALABAMA: Cullman, Cullman Co.; Ross and Ross; Jan. 12, 1963, 1 ♂. 5 miles NE of Cullman, Cullman Co.; Ross and Ross; Jan. 12, 1963, 2 ♀. 2 miles W of Attalla on US 431, Etowah



Figures 5–7. *Taeniopteryx metequi.*

FIG. 5. Male terminalia from below, with tip of 9th sternite pressed forward to expose the subanal lobes.

FIG. 6. Male terminalia from the side.

FIG. 7. Female terminalia from below.

Co.; H. B. Cunningham; Dec. 28, 1965, 2 ♂. Pelham on US 31, Shelby Co.; H. B. Cunningham; Jan. 14, 1966, 3 ♂, 2 ♀. 10 miles NE of Jasper on Route 69, Walker Co.; Ross and Ross; Jan. 12, 1963, 2 ♂, 2 ♀.

ARKANSAS: Osage Cr., Benton Co.; L. O. Warren; Jan. 29, 1962, 1 ♂. Cove Cr., SW 1/4, Sec 24, T 12N, R 27W, Franklin Co.; Hite and Aggus; Jan. 9, 1963, 1 ♂. S Fork White Oak Cr., SW 1/4, Sec. 17, T 10N, R 27W, Franklin Co.; Hite and Aggus; Jan. 9, 1963, 1 ♀. Coleman Cr., Hwy 7, Garland Co.; Hite and Wall; March 9, 1963, 1 ♀. King's R., Madison Co.; L. O. Warren; Jan. 26, 1962, 1 ♀. Maxwell Cr., Hwy 21, Kingston, Madison Co.; Hite and Wall; March 8, 1963, 1 ♂. Big Maumelle R., Hwy 10, Perry Co.; Hite and Wall; March 9, 1963, 1 ♀. Mill Cr., Polk Co.; L. O. Warren; Feb. 17, 1962, 1 ♂, 1 ♀. Five

miles N of Wickes, Polk Co.: Ross and Stannard; Feb. 10, 1959, 2 ♀. Ross Cr., 2 miles S of Waldron on Hwy 71, Scott Co.; Ross and Stannard; Feb. 10, 1959, 1 ♂. 3 miles NE of Williford, Sharp Co.; Ross and Stannard; Feb. 18, 1962, 1 ♂.

ILLINOIS: Lusk Cr., S of Eddyville, Pope Co.; Ross and Stannard; March 5, 1958, 8 ♂, 6 ♀. Bell Smith Springs, NW of Golconda, Pope Co.; Evers and Stannard; March 14, 1961, 2 ♂, 1 ♀.

INDIANA: S of Paoli, Orange Co., Frison and Mohr; Feb. 14, 1938, 2 ♀.

KANSAS: Shoal Cr. near Galena, Cherokee Co.; S. D. Smith; Jan. 16, 1968, 1 ♂, 6♀.

KENTUCKY: East Fork, West Catlettsburg, Boyd Co.; Ross and Ross; March 10, 1959, 2 ♂, 2 ♀. Hanging Branch, E of Danville, Boyle Co.; W. E. Ricker; March 10, 1964, 1 ♀. Rough R., S of Madrid, Breckenridge–Grayson Co. line; Rotramel and Unzicker; March 3, 1966, 1 ♂. Rockcastle R., 10 miles S of McKee, Jackson Co.; J. M. Campbell; Feb. 2, 1964, 3 ♂, 2 ♀. Muddy Cr., Waco, Madison Co.; L. J. Stannard; March 11, 1964, 1 ♂. Buck Cr., Stab, Pulaski Co.; L. J. Stannard; March 12, 1964, 1 ♀. Renfro Cr., Renfro Valley, Rockcastle Co.; Ross and Allen; December 26, 1964, 1 ♂. Cumberland R., 7 miles NW of Siler, Whitley Co.; Braasch and Thomas; Jan. 27, 1965, 1 ♂. Junction of Roads 25 and 26, 2 miles N of Williamsburg, Whitley Co.; Ross and Allen; Dec. 26, 1964, 17 ♂, 6 ♀. Poplar Cr., 2 miles NW of Siler, Whitley Co.; Braasch and Thomas; Jan. 25, 1965, 3 ♀.

MARYLAND: 10 miles E of Cumberland; R. E. Yeatter; Feb 13, 1938, 2 ♀.

MISSOURI: Hurricane Cr., Marble Hill, Bollinger Co.; Smith and Stannard; Feb. 10, 1961, 1 ♂, 1 ♀. N Fork White R., Twin Bridges, Douglas Co.; Rotramel and Rotramel; Jan. 6, 1966, 1 ♂.

NEW YORK: Tributary of Grindstone Creek, at Route 11, 1 mile S of Pulaski, Oswego Co.; L. L. Pechuman; April 2, 1964, 5 ♂, 1 ♀.

NORTH CAROLINA: Eno R., Durham, Durham Co.; R. D. Cuyler; Jan. 14, 1963, 1 ♀; Feb. 13, 1963, 6 ♂, 1 ♀; Mar. 3, 1963, 2 ♂, 1 ♀. Eno R., 5 miles N of Durham, Durham Co.; Wray and Greene; Jan. 16, 1962, 1 ♀. Eno R., on Hwy 501 N of Durham, Durham Co.; Wray and Greene; March 16, 1962, 1 ♀. Tar R., at Hwy 158, 1 mile W of Berea, Granville Co.; J. F. Greene; Jan. 17, 1963, 1 ♂, 1 ♀. Lower Little R., Harnett Co.; Feb. 7, 1937, 1 ♀. Morgan Creek, Chapel Hill, Orange Co.; R. D. Cuyler; Feb. 14, 1963, 4 ♂, 2 ♀. University Lake, Orange Co.; R. D. Cuyler; Jan. 17, 1962, 3 ♂, 1 ♀; Feb. 3, 1963, 1 ♀. Creek E of Roxboro on Hwy 158 at Surle, Person Co.; Wray and Greene; Jan. 17, 1963, 3 ♀. Black Creek on Road 50, Sampson Co.; J. F. Greene; March 18, 1958, 1 ♀. Small stream on Hwy 49, Mt. Misenheimer, Stanly Co.; J. F. Greene; Feb. 13, 1962, 1 ♀. Creek 1 mile W of Bayleaf, Wake Co.; Wray and Greene; Jan. 8, 1965, 2 ♂. Horse Creek, Hwy 98 near Wake Forest, Wake Co.; Harris and Wray; Jan. 22, 1964, 1 ♀. Neuse R., Hwy 50 N, Wake Co.; Wray and Greene; Mar. 6, 1958, 1 ♀; Jan. 21, 1964, 1 ♂. Neuse R., at Hwy 15 N of Raleigh, Wake Co.; Wray and Greene; Jan. 8, 1965, 1 ♂. Neuse R., at NW tip of Wake Co. 2 miles from Redwood; Wray and Greene; Feb. 7, 1964, 1 ♂.

OKLAHOMA: Fourche Maline R., Wilburton, Latimer Co.; Ross and Ross; Feb. 10, 1961, 2 ♂. Five miles E of Big Cedar, Leflore Co.; Ross and Ross; Feb. 11, 1961, 2 ♂, 3 ♀. Big Cedar Creek, Whitesboro, Leflore Co.; Ross and Ross; Feb. 11, 1961, 2 ♀. Bohannon Creek, Whitesboro, Leflore Co.; Ross and Ross; Feb. 11, 1961, 6 ♂.

ONTARIO: Bronte Creek at Carlisle, NE of Hamilton, Wentworth Co.; D. M. Wood; March 28, 1960, 2 ♂.

PENNSYLVANIA: 4.5 miles N of Warfordsburg, Fulton Co.; Hensley and Smith; Mar. 14, 1965, 1 ♂, 2 ♀. Beaver Spring, Snyder Co.; Unzicker and Allen; Jan. 29, 1966, 4 ♂ 2 ♀.

TENNESSEE: 3 miles W of Crab Orchard, Cumberland Co.; Braasch and Thomas; Jan. 29, 1965, 1 ♀. 15 miles E of Crossville, Cumberland Co.; H. B. Cunningham; March 11, 1964, 1 ♂. 8 miles SE of Mayland, Cumberland Co.; Braasch and Thomas; Jan. 29, 1965, 1 ♂, 2 ♀. Obed R., 2 miles N of Crossville, Cumberland Co.; H. B. Cunningham; March 11, 1964, 40 ♂, 43 ♀. 2 miles N of Crossville, Cumberland Co.; H. B. Cunningham; March 11, 1964, 1 ♂. 5 miles S of Crossville, Cumberland Co.; H. B. Cunningham; March 12, 1964, 1 ♂. Rock Cr., Pilot Mountain, Morgan Co.; Ross and Allen; Dec. 30, 1964, 3 ♂,

1 ♀. Sunbright Cr., Sunbright, Morgan Co.; Ross and Allen; Dec. 30, 1964, 10 ♂, 7 ♀. New R., Rugby, Morgan Co.; Ross and Allen; Dec. 30, 1964, 14 ♂, 6 ♀. Clear Fork R., Route 52, Morgan and Fentress Co.; Ross and Allen; Dec. 30, 1964, 5 ♂, 3 ♀.

VIRGINIA: Gore, Frederick Co.; Ross and Ross; March 4, 1959, 1 ♀. Big Walker R. at Route 100, Gilles Co; S. E. Neff; Jan. 14, 1965, 1 ♂. Bull Run, 6 miles E of Gainesville, Prince William Co.; Hensley and Smith; March 16, 1965, 2 ♂, 5 ♀.

WEST VIRGINIA: Elk R., 5 miles E of Clay, Clay Co.; Unzicker and Allen; Jan. 25, 1966, 1 ♀. Victor, Greenbrier Co.; Unzicker and Allen; Jan. 25, 1965, 1 ♂, 3 ♀. 4.5 miles E of Weston, Lewis Co.; Hensley and Smith; March 17, 1965, 1 ♂. 1 mile S of Boyer, Pocahontas Co.; Unzicker and Allen; Jan. 26, 1966, 1 ♂, 1 ♀.

Taeniopteryx nivalis (FITCH 1847)

1847. *Nemoura nivalis* Fitch.
1925. *Taeniopteryx nivalis* Needham and Claassen (in part).
1942. *Taeniopteryx maura* Frison (in part, not Pictet, 1841).
1944. *Taeniopteryx* sp., cf. *maura* Ricker.
1945. *Taeniopteryx maura* Ricker (in part: includes part of the specimens from Warsaw, Indiana).
1952. *Taeniopteryx maura* Harden and Mickel (at least in part — most of these records pertain to *nivalis*, but a few may be *burksi*).
1959. *Taeniopteryx maura* Jewett.
1960. *Taeniopteryx maura* Jewett.

Fitch's specimens are gone, but his minute description easily identifies his material as a *Taeniopteryx*. The statement "male organ exserted, forming a conical lurid point near the base of the last ventral segment" indicates the presence of a ventral lobe or vesicle on the 9th sternite. From the statement "femurs cylindrical, black, clothed with white hairs, which are longer and more distinct in the females, inner side with a narrow deep groove which is dilated toward the apex" it is clear that Fitch had examined male femora and that he would have seen and mentioned spurs if they had been present; hence he was not dealing with *maura*. Another vesicle-bearing *Taeniopteryx* (here called *burksi*) is present in eastern New York though apparently much less common, and it might have been included by Fitch in his series. However, it is clear that he had at least some of the type described below, for he says that *nivalis* begins to emerge during the latter half of winter "soon after the Small Snowfly [*Allocapnia*] is first met with," and that it occurs with the latter on the snow; *T. burksi* begins to emerge later in the season than *nivalis* in regions where both species occur. The use of the name *nivalis* proposed here also agrees with the Needham and Claassen illustration of female *nivalis* (Plate 46, fig. 8), which is clearly of this species. The diagnostic characters are as follows.

MALE: Hairs near the hind margin of the 9th sternite usually shorter than those farther forward on the sternite; when as long (as in some Quebec specimens), they are directed strongly downward and even somewhat forward (anteriorly). Aedeagus, when extruded, sclerotized lightly at the inside angles of the lateral bulbs, but lacking a strong sclerotized band across the central bulb (sometimes slightly greyish in that region, usually white). Vesicle of the 9th sternite 2–3 times as long as wide, hence shorter than in *burksi* and *maura*. Hind femora lacking any trace of spurs or swelling near the middle.

FEMALE: Sides of the median notch of the 8th sternite forming an acute angle medially (if produced forward), heavily sclerotized and having sharply rounded "shoulders" where they turn to the sides of the sternite (Needham and Claassen, 1925, Plate 46, fig. 8; Ricker, 1944, fig. 1).

The records below indicate a northern distribution for *nivalis* in the east, from Labrador through Quebec and Ontario to Minnesota; the southern limit is in New England, Pennsylvania, northern Indiana and Illinois, and southern Minnesota. This range broadly overlaps the northern part of *burksi*, while *maura* occurs with both in the Maritime Provinces and rarely elsewhere. *Taeniopteryx nivalis* has often been collected from the same streams as *burksi*, tending to emerge earlier in the season than the latter, though with much overlap. Intergradation between *nivalis* and *maura* has not been observed in either male or female characters. In the cordillera *nivalis* is rather uncommon, though known from northern California to Idaho. Although there are no clear distinguishing characters that would warrant separating them as a distinct subspecies, cordilleran specimens seem to be a homogeneous group, rather small in average size. The Alberta female is more like eastern specimens, and suggests that range of the species may be continuous across the continent near the northern margin of the grasslands.

Dr Frison's two Illinois papers (1929, 1935) are not listed in the synonomy above because there is no evidence he had any *nivalis* specimens from that state, where the species is evidently rare; at any rate none of his specimens still in the INHS collection are of this species.

ALBERTA: Bigoray R., 53°24'N, 115°05'W, Hugh F. Clifford, April 25, 1967, 1 ♀.
CALIFORNIA (Feb. 10): Siskiyou Co. (Jewett, 1960).
CONNECTICUT (March 15–April 7): Fairfield (1), New Haven (3).
IDAHO: Palouse R., Laird Park, Latah Co. S. D. Smith, April 10, 1962, 3 ♀.
ILLINOIS (March 6–12): De Kalb (1), Kendall (1), La Salie (1).
INDIANA (March 9): Kosciusko (2).
MAINE: Penobscot (1).
MICHIGAN (February 25–April 25): Arenac (1), Cheboygan (1), Clinton (1), Crawford (2), Ingham (4), Kalkaska (1), Lake (1), Midland (1), Presque Isle (1), Schoolcraft (1), Washtenaw (3).
MINNESOTA (March 20–April 11): Cooke (1), Lake (1), St. Louis (1), Wabasha (1).
NEW BRUNSWICK (April 13–15): Kent (2), Northumberland (5), Queens (4), York (2).
NEWFOUNDLAND AND LABRADOR (June 12): Hopedale (Ricker, 1944).
NEW HAMPSHIRE (March 22–April 2): Hillsboro (1).
NEW YORK (February 26–April 13): Broome (3), Chenango (1), Cortland (2), Delaware (4), Erie (1), Fulton (1), Herkimer (1), Jefferson (3), Lewis (1), Madison (2), Monroe (1), Niagara (2), Oneida (1), Onondaga (1), Ontario (1), Orange (1), Oswego (1), Otsego (12), Steuben (2), Tioga (4), Tompkins (8).
NOVA SCOTIA (April 15–19): Colchester (2), Cumberland (1), Guysboro (1), Halifax (2), Inverness (1), Pictou (1), Victoria (1).
ONTARIO — *north* (April 12): Thunder Bay (11).
ONTARIO — *south* (March 15–April 14): Carleton (2), Dufferin (1), Grey (2), Halton (2), Hastings (1), Peel (1), Waterloo (2), Wellington (8), Wentworth (1).
OREGON (January 1–March 19): Benton (1), Multnomah (2).

PENNSYLVANIA (March 3–April 19): Bradford (2), Cameron (1), Columbia (1), Dauphin (1), Lackawana (1), Lycoming (1), McKean (3), Potter (1), Snyder (1), Sullivan (3), Susquehanna (1), Tioga (2), Union (1).

QUEBEC (March 28–May 3): Champlain (1), Charlevoix (2), Chicoutimi (1), Gatineau (3), Joliette (1), Laviolette (3), Montmorency (1), Montmorency: Ile d'Orléans (6), Portneuf (2), Quebec (1), Timiskaming (1).

VERMONT (April 4): Bennington (1).

WISCONSIN (March 10–April 29): Dane (1), Oconto (2), Sauk (1), Shawana (1), Vilas (3), Washburn (1).

Taeniopteryx parvula BANKS 1918

1918. *Taeniopteryx parvula* Banks. Described from Virginia and the District of Columbia.
1925. *Taeniopteryx parvula* Needham and Claassen.
1929. *Taeniopteryx parvula* Frison (in part). See under *lita* for specimens wrongly identified.
1935. *Taeniopteryx parvula* Frison (in part). See under *lita*.
1942. *Taeniopteryx parvula* Frison. Selects lectotype from Peach Grove Hill, Virginia.
1952. *Taeniopteryx parvula* Harden and Mickel.

Needham and Claasen's illustration of male *parvula* (Plate 44, fig. 1) is unmistakeable, but that of the female (Plate 46, fig. 9) is difficult to relate definitely to any particular species. Their illustration captioned female *maura* (Plate 46, fig. 7), however, may be *parvula*. Frison's illustration (1929, fig. 77; 1935, fig. 214) is good in showing the unsclerotized central portion of the 8th sternite, but the two inward-pointing horns of the central sclerotized plate are not evident in any specimens we have examined.

Frison (1942, p. 250) has suggested that some of the Needham and Claassen *parvula* records might actually be *lita*, and confusion with the new species *metequi* would be even more likely. We have examined the Needham and Claassen specimens from Wisconsin and Maine, and part of those from the District of Columbia, and have confirmed their identification as *parvula*; two others (from Massachusetts and Minnesota) are outside the known range of *lita* and *metequi*, so are almost certainly *parvula*. Needham and Claassen specimens from Maryland and New Jersey have not been rechecked, and more recent specimens from those states are not available, though they lie within the probable range of *parvula*.

Needham and Claassen erroneously quote Plummer's Island, Maryland, as the source of the holotype and allotype of *parvula*; no specimens from this locality are mentioned in Banks's description.

The records below show that *T. parvula* is centred about the Great Lakes region, extending beyond the lakes as far as Cape Breton Island on the east, to Hudson Bay on the north, and to Manitoba on the west. In the Appalachian region *parvula* extends south to Georgia; in the Mississippi valley there is a gap from the Ohio River to the Ozark region of southern Missouri and Arkansas.

ARKANSAS (January 26–March 9): Montgomery (2), Saline (1), Sharp (1), Washington (1), Yell–Conway (1).

DISTRICT OF COLUMBIA (February 15–March 12): (2).

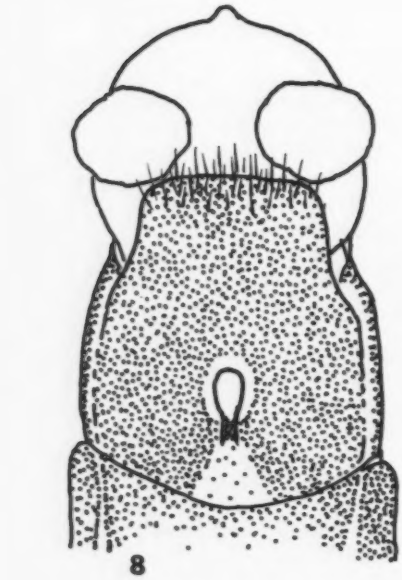
GEORGIA: 22 miles E of Hiawassee, 4 miles W of Clayton, Rabun Co.; Stannard and Brooks; January 30, 1963, 1 ♂.
ILLINOIS (March 7–April 23): Hardin (1), Lee (1), Ogle (1), Winnebago (1), Whiteside (1).
INDIANA (February 4–March 6): Dubois (1), Greene (1), Monroe (1).
MAINE (March 24): Penobscot (1).
MANITOBA: Aweme, near Treesbank, 20 miles SE of Brandon; N. Criddle; April 11, 1928, 1 ♂.
MICHIGAN (March 1–April 25): Grand Traverse (1), Houghton (1), Kalkaska (1).
MINNESOTA (April 5–11): Cook (1), Lake (4), St. Louis (2).
MISSOURI (February 11–17): Pulaski (1), Texas (1).
NEW BRUNSWICK (April 13–15): Kent (5), Northumberland (5), Queens (4), Sunbury (1), Westmorland (5), York (3).
NEW HAMPSHIRE (March 13–16): Hillsboro (1), Merrimack (3).
NEW YORK (March 17–April 3): Broome (1), Essex (2), Hamilton (10), Herkimer (4), Niagara (1), Oneida (1), Oswegatchie (1), St. Lawrence (3), Steuben (1).
NORTH CAROLINA (January 13–April 3): Chatham (1), Durham (1), Jackson (2), Johnston (2), Macon (2), Nash (1), Wake (3).
NOVA SCOTIA (April 15–20): Colchester (2), Cumberland (1), Guysboro (3), Halifax (1), Lunenburg (1).
ONTARIO — *south* (March 16–April 6): Hastings (1), Renfrew (1).
ONTARIO — *north* (April 6–19): Nipissing (3), Thunder Bay (7). Also Winisk, Patricia Dist., May 1, 1956, V. Crichton, 1 ♂.
PENNSYLVANIA (March 14–16): Cameron (1), Dauphin (1), McKean (1), Tioga (1).
QUEBEC (April 2–23): Charlevoix (3), Chicoutimi (2), Gatineau (2), Joliette (1), Labelle (1), Laviolette (2), Lotbinière (1), Montcalm (1), Montmorency (3), Montmorency: Ile d'Orléans (1), Portneuf (2), Quebec (3), Terrebonne (1), Timiskaming (1).
RHODE ISLAND (March 3–7): Washington (1).
TENNESSEE: Spring Creek, Montgomery Co., F. M. Ford, March 10, 1963, 1 ♀.
VIRGINIA (January 23–February 21): Alleghany (1), Montgomery (1), Roanoke (1), Village of Peach Grove Hill.
WEST VIRGINIA (January 25): Clay (1).
WISCONSIN (March 12–April 10): Barron (1), Jackson (2), Marinette (1), Trempealeau (2).

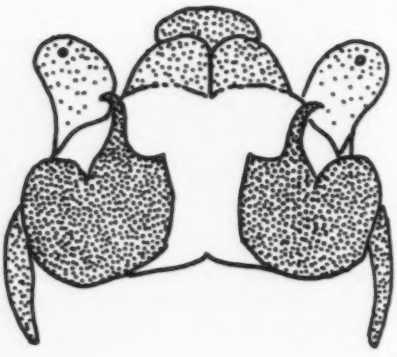
Taeniopteryx ***ugola*** n.sp.

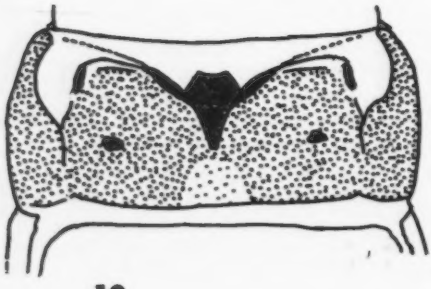
MALE: Length of body 8 mm, to tip of wings 8 mm. Colour dark brown in alcohol, black in life. Epiproct similar to *nivalis* and other species of the genus. Cerci of 1 segment, somewhat excavated on the outer surface. Ninth sternite with a well-developed lobe about 3 times as long as wide, and long terminal hairs (Fig. 8). Subanal lobes with tips slender, subacute, curved outward (Fig. 9), much as in *parvula*. Aedeagus (Fig. 8) lacks sclerotization, though there is (in some lights) a darkening of the integument in a narrow band ventrally joining the lateral bulbous processes.

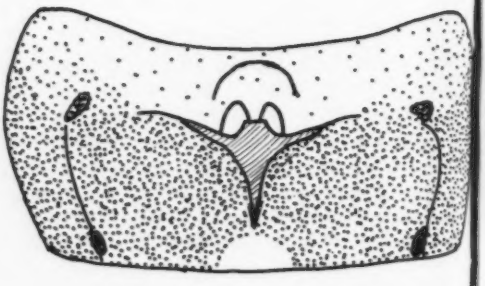
FEMALE: Length of body 10 mm, to tip of wings 11.5 mm. Eighth sternite normally sclerotized, the median plate dark, rather broad, slightly excavated at the tip; the "shoulder" of the V heavily sclerotized for a short distance (Fig. 10).

The male of this species very closely resembles *parvula* except that the latter lacks a ventral lobe on the 9th; also, the hooks of the subanal lobes are a little more slender and sharper in *parvula*. The female is closest to *burksi*, but the broad median plate and the extra sclerotization of the shoulder of the V-line are distinctive. The name *ugola* refers to the coal measures that underlie the home of this species.









Figures 8–10. ***Taeniopteryx ugola.***

FIG. 8. Male terminalia from below, with extruded aedeagus.

FIG. 9. Male 10th segment from below (9th sternite removed).

FIG. 10. Female 8th sternite.

Figure 11. ***Taeniopteryx burksi.***

FIG. 11. Female 8th sternite.

Holotype ♂, allotype ♀, 3 ♂ and 2 ♀ paratypes, Fellowsville, Preston Co., West Virginia, March 4, 1959, H. H. Ross and J. A. Ross. The same, at Sandy Creek, 1 ♂. Other paratypes as follows.

TENNESSEE: (except where noted, all collections were made by H. B. Cunningham): 8 miles SE of Maryland, Cumberland Co.; Braasch and Thomas; Jan. 29, 1965, 1 ♂, 1 ♀. Obed R., 2 miles N of Crossville, Cumberland Co.; 1 ♂. 5 miles N of Crossville, Cumberland Co., March 11, 1964, 1 ♂. 2 miles S of Crossville, Cumberland Co.; March 12, 1964, 6 ♂, 4 ♀. 8 miles S of Crossville, Cumberland Co.; March 12, 1964, 1 ♂, 1 ♀. Small sandy creek, Sumner City, Sumner Co.; March 12, 1964, 3 ♂, 2 ♀. Creek on mountain, Cumberland Heights; March 13, 1964, 11 ♂, 10 ♀. Creek on mountain, S edge of Cumberland Heights; March 13, 1964, 1 ♂.

WEST VIRGINIA: Flattop (on turnpike), Mercer Co.; Ross and Ross; March 10, 1959, 1 ♂.

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Fish Attraction with Pulsed Low-Frequency Sound[1,2]

By Joseph D. Richard

Institute of Marine Science
University of Miami, Miami, Florida, U.S.A.

ABSTRACT

A series of tests were conducted to determine the effectiveness of pulsed low-frequency acoustic signals for attracting fishes. The acoustic signals were contrived to simulate the hydrodynamically generated disturbances normally associated with active predation. Underwater television was used to observe fish arrivals during both control and test periods. Demersal predatory fishes were successfully attracted although they habituated rapidly to the acoustic stimulus. Members of the families Serranidae, Lutjanidae, and Pomadasyidae were particularly well represented among the fishes attracted. Sharks were also attracted in considerable numbers. Herbivorous reef fishes, although common around the test site, were not attracted. Possible relationships between the test results and the hearing capabilities of fishes are discussed. It is concluded that acoustic attraction techniques have potential applications in certain existing commercial fisheries.

INTRODUCTION

It is generally well known that fishes are able to both detect and generate acoustic and other mechanical disturbances in their surrounding medium. When information is conveyed by such disturbances, they are usually referred to as acoustic signals. Many fish possess a specialized mechanism for producing acoustic signals characteristic of their species. Biologically significant acoustic signals may also be radiated hydrodynamically by the rapid acceleration (or by abrupt or convulsive movements) of the fish's body. Whatever the source, localized water displacements and propagated compressional waves both result when the medium is thus disturbed. Although these two components of a radiated mechanical disturbance co-exist near their source, they differ greatly in their attenuation characteristics so that only the propagated compressional waves extend out an appreciable distance. Although a considerable literature is available relating to hearing and allied senses in fishes (mostly based on laboratory experiments), very little is known about how they respond to specific acoustic signals when in their natural habitat. Presumably, acoustic signaling (both active and passive) plays an important role in many of their predatory–prey, and other, behavioral interactions (Griffin, 1955; Malyukina and Protasov, 1960; Moulton, 1963; and Tavolga, 1965).

The sensory mechanisms by which fishes detect mechanical disturbances in the near- and far-field and the functional relationship of lateral-line organ and internal auditory apparatus have been described by Lowenstein (1957), Harris and van Bergeijk (1962), Dijkgraaf (1963), van Bergeijk (1964), and

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[2]Contribution No. 928 from the Institute of Marine Sciences, University of Miami, Miami, Fla.

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others. Considerations of the theory of hearing as evolved by these investigators has led to the conclusion that, although fishes hear in both the near- and far-field, they are capable of localization only in the near-field. As there is no conclusive experimental evidence to show that fishes can localize a sound source while in the far-field, behavioral observations which seem to indicate otherwise are generally considered questionable. The limiting distance encompassing the near-field depends on the acoustic wavelength and is variously defined as between $\lambda/2\pi$ and λ from the source, depending on the type of source. Greatly exaggerated displacement amplitudes in the near-field of a mechanical disturbance permit localization apparently with the lateral-line sensory system. Compressional waves are detected in the far-field, presumably without directional sense, by means of the internal auditory apparatus.

Although we know that fishes respond to sound in a variety of ways, very few experimental observations have been made of their acoustic behavior. Such observations have been difficult or impossible in the past because of the obvious viewing problems involved. However, in spite of the generally poor visibility through the air–water interface, startle responses have been observed very commonly among free-swimming fishes when an impulsive acoustic stimulus is introduced into the water. Although such responses are characterized by rapid habituation, many primitive fisheries use crude noise-making devices to drive fishes into nets or weirs (Anon, 1949; Kesteven, 1949; and many others). More recently, Moore and Newman (1956), Van Derwalker (1967), and others, have attempted to use an avoidance response to artificially generated sounds of high intensity as a means for driving salmon away from turbine intakes and into alternate channels.

From the limited available evidence, it seems likely that acoustic attraction plays a significant role in the mating behavior of at least some fish species (Moulton, 1963). Sounds associated with prey are almost certainly an effective attractant to many predatory fishes. Laboratory experiments have repeatedly demonstrated that fish are easily conditioned to approach a sound source to obtain a food reward. Needham (1954) reported the use of artificially generated feeding sounds for concentrating pond-reared fish for harvesting. The water-spray technique used by the Japanese skipjack fisheries (Anon, 1948) simulates acoustically (and visually) the surface agitation normally associated with active feeding and this is apparently an effective attractant. Acoustic attraction techniques are used in many primitive fisheries although generally the biological significance of the particular sounds used remain unknown (Westenberg, 1953; Busnel, 1959; von Brandt, 1964; and Wolff, 1966). However, until recently (Wisby and Richard, 1963; Wisby et al., 1964; and Hashimoto and Maniwa, 1967) there have been very few experimental observations of fishes being spontaneously attracted to an artificially generated acoustic stimulus.

This paper presents the results of a recent series of tests which show that acoustic attraction techniques can be very effective for at least some fish species. These techniques, particularly if coupled with the use of bait as reinforcement, may be applicable to certain commercial fisheries in the near future.

EXPERIMENTAL PROCEDURES

Figure 1 shows the experimental arrangement used for observing the responses of free-swimming fishes to acoustic stimuli. The observation system consisted of a television monitor (located in the Lerner Marine Laboratory,

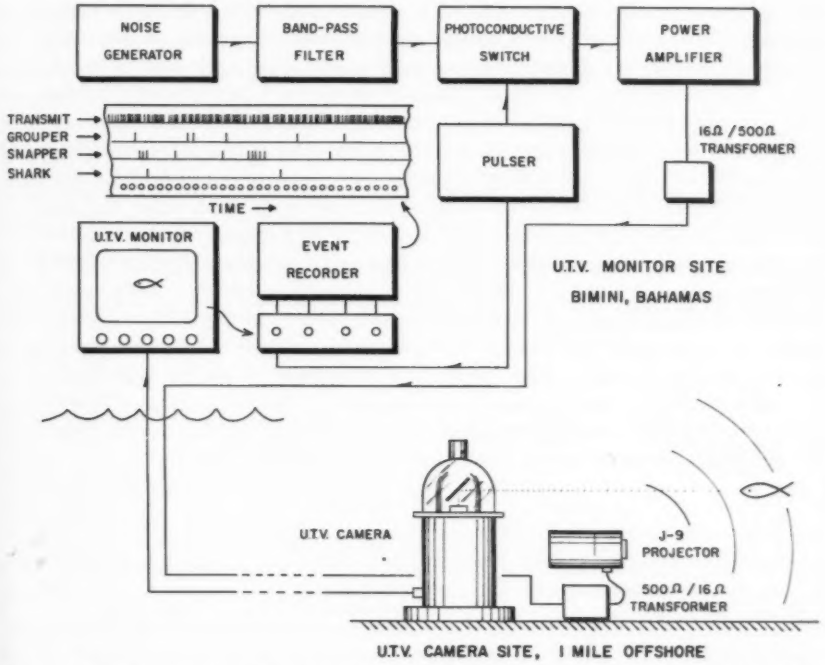

FIG. 1. Experimental apparatus for observing the responses of fishes to acoustic stimuli.

Bimini, Bahamas) and connecting underwater television camera mounted on the bottom in 20 m of water approximately 1.5 km offshore to the west. This underwater video system has been described previously (Kumpf and Loewenstein, 1962; Kumpf, 1964; Kronengold et al., 1964; and Steinberg et al., 1965). Acoustic transmission equipment consisted of random noise source, variable filter, pulse-forming circuitry, power amplifier, and impedance matching transformer in the monitor laboratory. A second impedance matching transformer and J-9 acoustic projector were mounted adjacent the television camera on the bottom. Pulsed acoustic signals were transmitted into the water adjacent the television camera using the equipment shown in Fig. 1. Principal variables in the signal characteristic were frequency spectra, modulation envelope, and source level. For most of the tests reported here, the signal consisted of an octave band of noise having lower and upper cut-off frequencies of 25 and 50 Hz respectively, and lower and upper attenuation

slopes of 24 db per octave. Other signals used included a higher frequency octave noise band and complex sounds abstracted from tape recordings. Generally, randomly spaced pulses varying in repetition rate from several pulses per minute to several per second were transmitted during the test periods. Pulses of constant duration and repetition rate were transmitted during only a few test periods. The pulse characteristic and frequency content of the transmitted acoustic signals were contrived to simulate the irregular hydrodynamic disturbances generated by actively feeding predatory fishes. Source spectrum levels of about 20 db above 1 microbar (1 m from the source) were used for most of these tests. If it is assumed that fishes are able to detect these acoustic signals out to a limiting distance where they fall below the ambient noise levels, a maximum range of audibility of approximately 100 m might be expected under typical sea state conditions. If, however, a threshold/noise relationship based on actual test results from the literature is assumed (wherein thresholds are reported to be substantially above noise), a maximum range of audibility of only 50 m, or even considerably less, might be expected. In either case, the bottom area over which the signal was presumably audible was adequate for testing purposes as these acoustic signals are propagated omnidirectionally. Test periods varied from 4 to 30 min and each was immediately preceded by a corresponding control period of the same duration. An event recorder was used to record all fish sightings while observing the television monitor during both control and test periods.

RESULTS

Figures 2–7 show, on a horizontal time scale, the order of arrival of various fishes into the field of view of the underwater television camera during individual

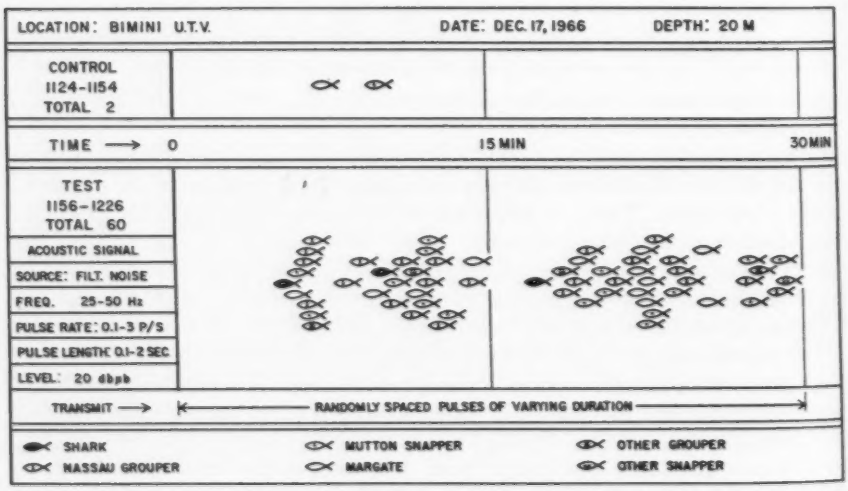

FIG. 2. Fishes observed during control and test periods on December 17, 1966. Acoustic signal: randomly pulsed noise, 25–50 Hz.

test periods and corresponding control periods. The fish symbols are positioned vertically in a completely arbitrary manner and the specific grouping in no way implies schooling structure. Many of the fishes observed may have been part of a widely dispersed school but, generally, this could not be established because of the restricted field of view. However, closely structured fish schools were observed during the tests illustrated by Fig. 3 and 5. Because individual fish of the same species are only rarely distinguishable, there is no way to avoid the recounting of individuals during a test period if they are re-attracted into the field of view.

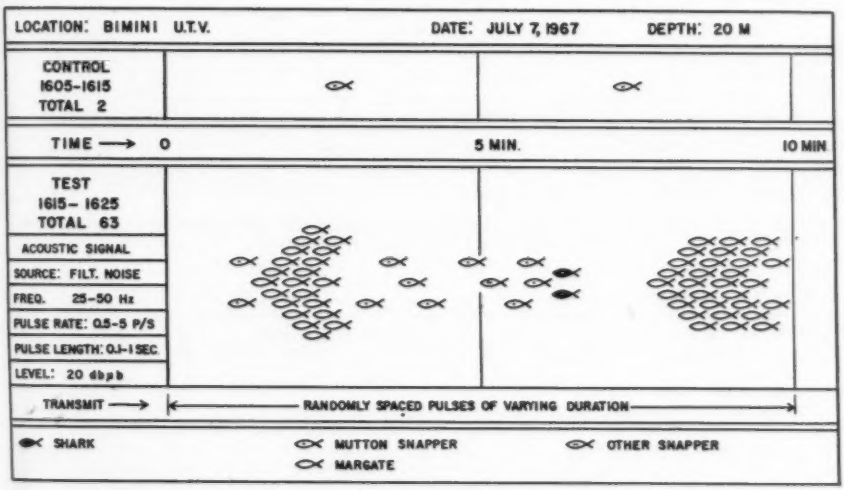

FIG. 3. Fishes observed during control and test periods on July 7, 1967. Acoustic signal: randomly pulsed noise, 25–50 Hz.

Each individual test reported here was originally part of a repetitive series of tests spaced in time only by a rest period of typically 5 min. However, only the first test of each series is shown here. Successive tests in a repetitive series produced rapidly diminishing results. This rapid habituation is characteristic of fishes when their responses to a stimulus remain unrewarded. This rapid habituation imposes severe limitations on the number of tests which can be conducted from a fixed site.

Figures 2–4 show the results of acoustic attraction tests in which the signal consisted of a randomly pulsed band of noise having cut-off frequencies of 25 and 50 Hz. Figure 5 shows results of a test using a similar signal except that it was pulsed regularly by means of a timing device. Figure 6 shows results of an acoustic attraction test using, in sequence, three types of complex sounds abstracted from field tape recordings. These signals were repeated about 10 times per minute by means of a magnetic tape loop. Acoustic output levels

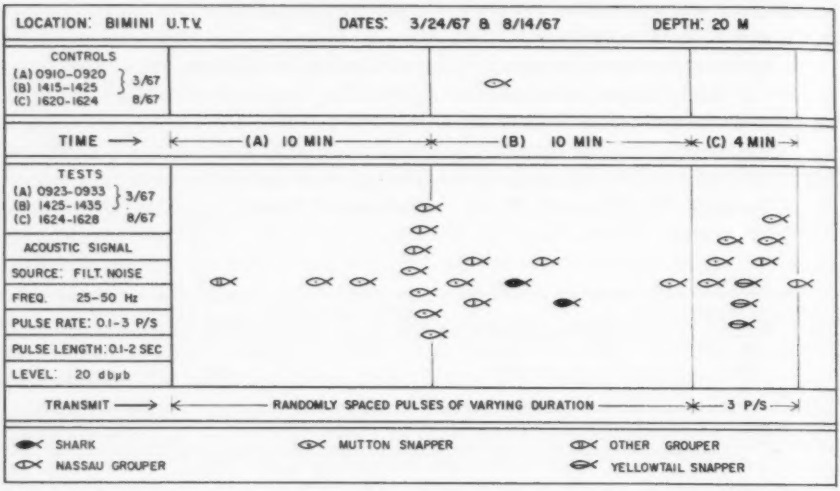

FIG. 4. Fishes observed during three separate test periods and corresponding control periods. Acoustic signal: pulsed noise, 25–50 Hz.

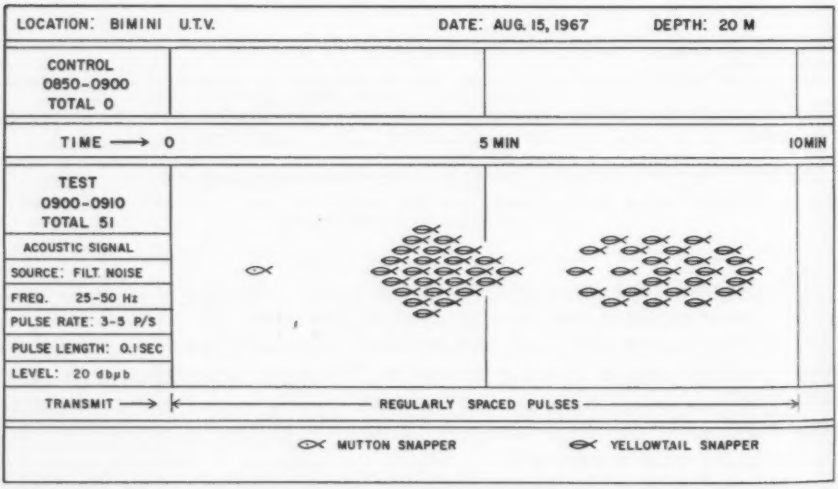

FIG. 5. Fishes observed during control and test periods on August 15, 1967. Acoustic signal: regularly pulsed noise, 25–50 Hz.

were estimated to be at least 40 db higher than the corresponding original sounds. Figure 7 shows the results of a test in which the signal consisted of a randomly pulsed band of noise having cut-off frequencies of 100 and 200 Hz.

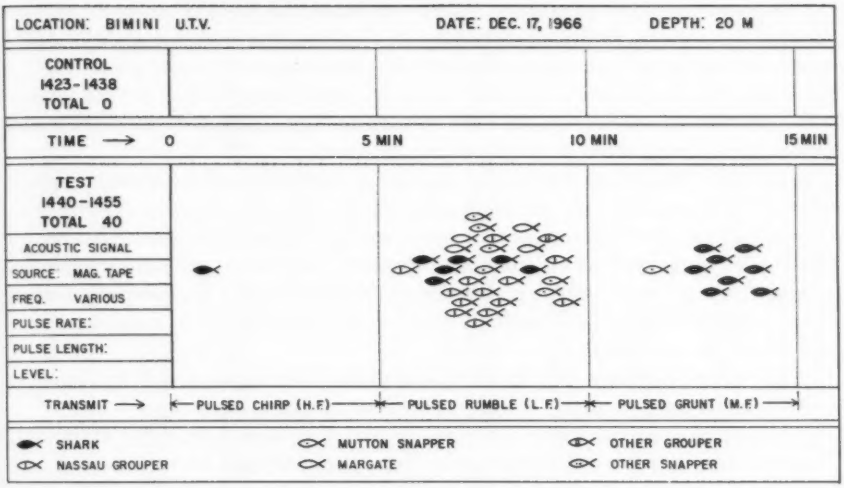

FIG. 6. Fishes observed during three consecutive test periods and corresponding control periods on December 17, 1966. Acoustic signal: complex sounds abstracted from magnetic tape recordings.

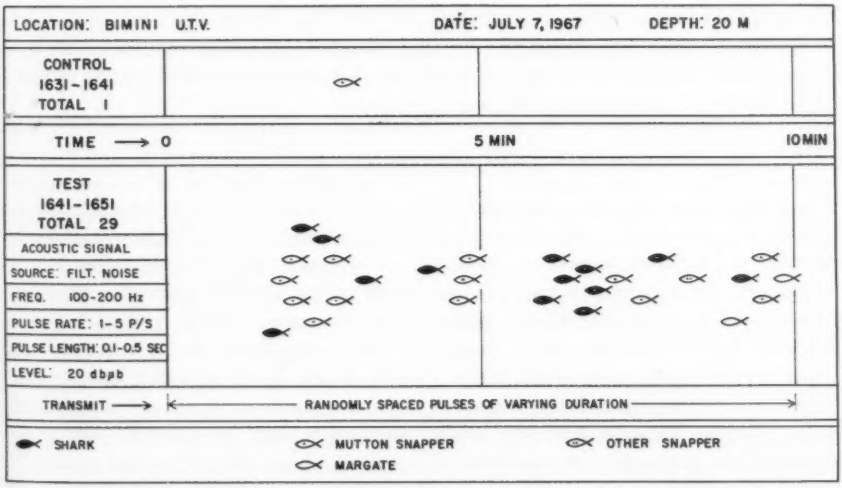

FIG. 7. Fishes observed during control and test periods on July 7, 1967. Acoustic signal: rapidly pulsed noise, 100–200 Hz.

Obviously, the relative effectiveness of these various types of acoustic signals cannot be evaluated using these limited data. Furthermore, seasonal, diurnal, and tidal differences between the tests, and the resulting variability in fish species and abundance, make any comparisons meaningless.

Apparently only predatory fishes are attracted by the acoustic signals used in these tests. Although abundant in the area, the herbivorous reef fishes were not attracted. Occasionally, startle responses were seen when one of these reef fish happened to be very near the sound source when the first pulse was transmitted. However, no such responses were observed as subsequent pulses were transmitted during a particular test.

Of the teleosts, the principal species attracted by the acoustic signals were (in the approximate order of abundance): Nassau grouper, *Epinephelus striatus* (Bloch); mutton snapper, *Lutjanus analis* (Cuvier); margate, (*Haemulon album* (Cuvier); yellowtail snapper, *Ocyurus chrysurus* (Bloch); yellowfin grouper, *Mycteroperca venenosa* (Linnaeus); black grouper, *Mycteroperca bonaci* (Poey). Also, several unidentified species of groupers and snappers were observed and counted.

Estimated average weights for these fishes were: groupers, 4 kg; mutton snappers, 1.5 kg; margates, 1 kg; and yellowtail snappers, 1 kg.

In addition to the above teleosts, sharks were also frequently attracted. Most commonly observed sharks were: sharpnose shark, *Rhizoprionodon sp.*; reef shark, *Carcharhinus* sp.; nurse shark, *Ginglymostoma cirratum.*

Figure 8 summarizes the results of the entire series of tests. Although no conclusions are drawn, there is some indication that relatively higher frequencies are more effective for attracting the sharpnose shark, *Rhizoprionodon sp.* Relatively higher frequencies were transmitted only for the second and sixth tests of the series, and both resulted in a high percentage of these sharks. An unknown factor here is the extent to which the teleosts are repelled by the sharks when both are attracted to the same location.

DISCUSSION

From these data it is apparent that low-frequency pulsed acoustic noise signals are an effective attractant for at least some predatory fishes. Although most of the tests reported here used an octave band noise signal having lower and upper cut-off frequencies of 25 and 50 Hz as the acoustic stimulus, positive results were also obtained with higher frequency noise bands and with more complex signals derived from magnetic tape recordings. Recent tests (not reported here) and also earlier tests (Wisby et al., 1964) have indicated that fishes are not attracted by either pulsed sine wave or constant amplitude noise signals. Apparently, substantial bandwidth and continuous amplitude modulation (pulsing) are essential characteristics of an attractive acoustic signal. However, one series of observations (Wisby and Richard, 1963) seemed to contradict this generalization. In these observations, yellowtail snapper (*Ocyurus chrysurus*) were consistently attracted using a 25-Hz oscillator signal pulsed directly into a power amplifier. More recently, it has been shown (unpublished data) that such signals can indeed be effective when the sine wave severely overdrives the power amplifier. The resulting clipped and squared waveform comprises a broad band of odd harmonics in addition to

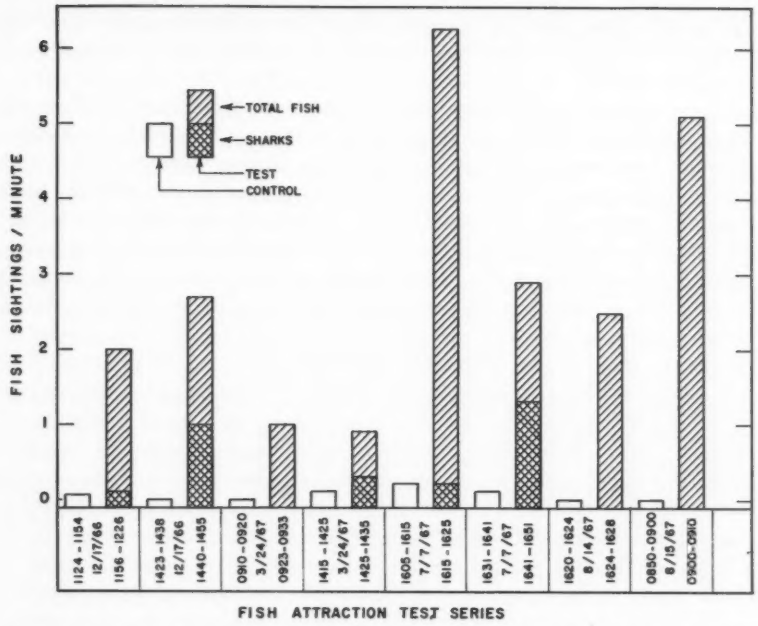

FIG. 8. Summary of test results showing the relative attraction of sharks and teleosts.

the fundamental frequency. Presumably, either the even harmonic series derived from triangular waves, or the odd and even series from saw-tooth waves, would be similarly effective.

Unfortunately, the signal waveforms used in these tests were not monitored on an oscilloscope. In attempting to maximize acoustic output power, it is likely that the power amplifier was overdriven by the octave band noise signals during some (if not all) of the tests. If so, a broad-band signal resulted which included an odd series of octave noise bands harmonically related to the fundamental noise band.

Although encouraging, the results of the tests reported here reveal very little about the relative effectiveness of different acoustic signals. Therefore, a comprehensive test series is planned which will delineate, if possible, the frequency spectra and pulse repetition characteristics which are most effective for attracting fishes. Possibly the optimum signal characteristics will vary significantly with species. Uncertainties in harmonic content will have to be eliminated in these tests, particularly for the very low-frequency signals. Because their hearing sensitivity increases rapidly with frequency (peaking around 400 Hz,) and because ambient noise levels decrease with frequency over this range, fishes may perceive one or more of the harmonics of a distorted signal even when the fundamental is inaudible. Another objective of future tests

will be to determine the optimum signal level for attracting fishes. Obviously, if the signal level is too intense, fishes will be repelled at some point as they approach the source.

Because of the variables involved, a relatively large number of tests will have to be made to resolve the question of optimum signal characteristics. As the present underwater television camera site is fixed in a relatively barren area, only a few species and small populations are subject to testing. Because the resident population of fishes living around the camera site habituate rapidly to the acoustic signals, only a few tests can be made before an extended rest period becomes necessary. It is expected that these disadvantages and limitations will be overcome in the future test series by the use of a portable underwater television system operated from a highly mobile houseboat.

From the foregoing, it is evident that substantial bandwidth and pulsing are two essential characteristics of an effective acoustic signal for attracting fishes. It is postulated that the impulsive character (pulse modulation) of the acoustic signal is the primary attracting component; probably because it simulates the noise bursts which are normally generated (hydrodynamically) by the rapid acceleration and abrupt maneuvers of actively feeding fishes. Probably the struggling of prey fishes upon capture also provides a brief but intense stimulation to the lateral line receptors of the predators involved. Although these hydrodynamically generated mechanical disturbances are essentially localized water displacements, they presumably are detectable by other nearby fishes. If a characteristic mechanical stimulus is normally received concomitantly with feeding activity, then presumably such a stimulus would be attractive to predators even in the absence of prey. Since the lateral-line and inner ear sensory receptors are closely related (and probably partially overlapping in function) it is possible that an artificially generated compressional wave, having similar modulation characteristics and frequency components, would also be an attractive stimulus even though detected by the inner ear in the far-field.

Although it is likely that fishes are able to detect and respond to an attractive compressional wave signal while in the far-field, it is generally assumed that they cannot localize the source of such a signal. Presumably, the fishes enter the near-field only through exploratory swimming and only then localize the source to make possible a direct approach. Since the extent of the near-field depends on the signal wavelength, the effectiveness of an attractive signal should be increased when very low-frequency components are included. On the other hand, higher frequency components are perceived more readily at a distance because of the increasing sensitivity of the inner ear (peaking at several hundred Hz) and a more favorable signal to ambient noise relationship. Thus to be effective over a wide area, an attracting signal might require both high- and low-frequency components.

CONCLUSIONS

It is concluded that acoustic signals of the character described can be effective for attracting at least some species of demersal predatory fishes.

These techniques should be applicable to other species having similar feeding habits. It is hypothesized that these attractive sounds simulate, in their pulse characteristics, the hydrodynamic disturbances normally associated with active predation. Low-frequency components are presumably essential to extend the near-field and permit localization of the source from a substantial distance. The effectiveness of acoustic attraction should be greatly enhanced if bait is used as a reinforcing stimulus. These acoustic signals may possibly contribute significantly to the initiation of feeding behavior among the fishes attracted. It is suggested that these acoustic attraction techniques are applicable to certain existing commercial fisheries such as the snapper and grouper fisheries in the Gulf of Mexico and Caribbean Sea areas.

ACKNOWLEDGMENTS

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Permanence of Station in Juvenile Chinook Salmon and Steelhead Trout[1]

By Eldon Edmundson,[2] F. E. Everest, and D. W. Chapman

Idaho Cooperative Fishery Unit, College of Forestry
University of Idaho, Moscow, Idaho, U.S.A.

ABSTRACT

Daytime movements and night locations of juvenile steelhead trout (*Salmo gairdneri*) and night locations of spring-run chinook salmon (*Oncorhynchus tshawytscha*) were observed in Johnson Creek and the Lochsa River in Idaho during the summers of 1965 and 1966. The observations were made by branding fish and observing them subsequently under water. The distances between sequential sightings of branded steelhead within each day and over longer periods were recorded. In 151 sequential sightings of steelhead within each day in Johnson Creek, 58% of the distances moved were less than 3 m and 86% were less than 6 m. In 59 sequential sightings of branded steelhead on successive days in the Lochsa River and Johnson Creek, 53% were less than 3 m and 68% were less than 6 m apart. In sightings of branded steelhead separated by 10–14 days, 45% (25) of the sequential sightings were less than 3 m and 63% were less than 6 m apart. Movements of spring-run juvenile chinook salmon within each day were measured in a stream aquarium (10.5 m long and 0.6 m wide). Of 272 observed daily movements, 65% were less than 0.6 m. Steelhead trout were inactive at night, occupying bottom areas of low velocity, usually inshore. During the day they were in areas of moderate current. Chinook salmon were observed at night to be both on the bottom and near the surface in quiet water, and on the bottom in flowing water. Chinook salmon were found at all depths in quiet water during the day. In winter, some steelhead trout were found in the substrate in the same area they occupied the previous summer.

INTRODUCTION

The purpose of this study was to learn if juvenile summer- and spring-run chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*) maintain a permanent stream station or home area.

Our specific objectives were to learn: (1) if juvenile chinook salmon and steelhead trout stayed in one home area throughout the summer; (2) whether the young fish occupied the same station or area during the day and night.

We are using the term "station" to mean a particular location at which a fish spends much of its time. It would be possible for a fish to have more than one station or none. By "home area" we mean the stream area over which a fish normally travels, excluding areas covered during migrations to and from the sea.

Field study was conducted in a segment of the Lochsa River and Johnson Creek in Idaho from June 1965 to November 1966. Additional research was done in a stream aquarium at the University of Idaho at Moscow, Idaho, in May 1966.

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[1]Received for publication June 14, 1967.
[2]Present address: Department of Zoology, Washington State University, Pullman, Wash.

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DESCRIPTION OF FIELD SITES

The study areas are within the drainage of the Snake River (Fig. 1), a tributary of the Columbia River.

One study site was a section of the Lochsa River, a major tributary of the Clearwater River, at a point 6.4 km below Powell Ranger Station. The river there was 21 m wide and up to 3 m deep. The study site was a section 6 m wide, 45 m long, and up to 1.2 m deep. The substrate was a mixture of sand, gravel, and rocks up to 1 m in diam.

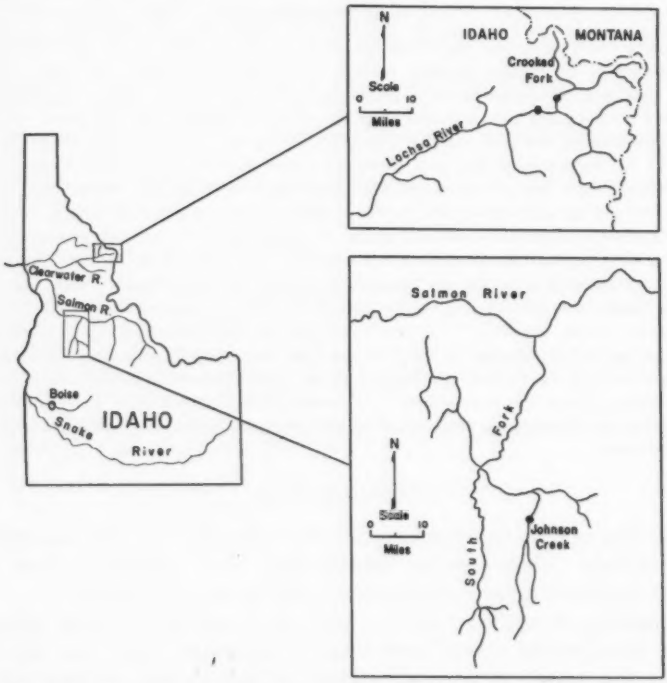

FIG. 1. Location of study sites on Johnson Creek and the Lochsa River.

The other study area was in Johnson Creek, which enters the East Fork of the South Fork of the Salmon River near the town of Yellow Pine, Idaho. The study area was 20 m wide, 66 m long, up to 1.2 m deep, and was located 6.4 km upstream from Yellow Pine. The bottom consisted of rocks up to 1 m in diam and sand and gravel.

Water temperatures, observed irregularly in all study areas during the summer of 1966, ranged from 10 to 18 C, with daily fluctuations of up to 8 C.

Fish present in the study areas were Dolly Varden (*Salvelinus malma*), brook trout (*S. fontinalis*), chinook salmon, steelhead trout, planted rainbow

trout (*Salmo gairdneri*), cutthroat trout (*S. clarki*), mountain whitefish (*Prosopium williamsoni*), and sculpins (*Cottus bairdi* and *C. rhotheus*). Dolly Varden, brook trout, planted rainbow trout, cutthroat trout, and mountain whitefish were not abundant during the study period.

Different times of emergence and seaward migration for chinook salmon and steelhead trout result in a gradient of sizes of juveniles found in the stream at any one time. Chinook salmon emerge in the spring and steelhead trout emerge during late July and August, when chinook salmon of the year range from 35 to 65 mm. Age I and older steelhead trout range from 90 to over 185 mm in late summer. Shortly after chinook salmon emerge, some downstream movement usually occurs, and there are larger migrations in the fall and following spring. Most steelhead trout juveniles have left natal streams by the spring of their 3rd or 4th year.

METHODS AND MATERIALS

Field Studies

Juvenile steelhead trout (65–185 mm) of ages I–III were used for the field study. They were caught by angling during the day and by divers with hand nets at night. Captured fish were measured to fork length, marked by cold-branding a two-digit number between the lateral line and dorsal fin (Everest and Edmundson, 1967), and released near the point of capture. After 5 days the brands were readable under water and remained so for 5–6 weeks. The numbers of branded juvenile steelhead released were: 49 into the Lochsa River in 1965, and 42 and 31 into Johnson Creek in 1965 and 1966 respectively. Releases were made on July 26 and 29, 1965 on the Lochsa River and on July 20, 1965 and June 28, 1966 on Johnson Creek.

Underwater observations were made by snorkeling and scuba diving starting several days after branding. Water visibility was usually about 20 m, with brands being readable at distances up to 3 m. Each dive began at the downstream end of the station, and observations were made for about 1 hr as the diver worked upstream. We moved slowly when in the water, making no abrupt movements, and found young salmon and steelhead paid us little heed. The fish moved out of our way as we approached too closely, then usually shifted back to previous positions as we passed on. Observations of the fish were between 0830 and 1000, 1300 and 1430, and 1700 and 1830 hr (Mountain Standard Time). This schedule prevented excessive diver fatigue. Occasional observations between scheduled dives led us to assume that our timing produced no serious biases in fish activity. Some observations were also made about 1 hr after darkness and 1 hr before sunrise.

We recorded the positions of identified fish on an underwater slate on which the stream bottom was represented by a grid system. Distance moved was taken as the straight-line distance between sightings.

Observations of fish in winter were made by divers using snorkeling and "primacord" explosives. A seine was placed downstream from the area to be

sampled to catch drifting injured fish and a diver carefully examined the blasted area after the detonation to collect fish lying on the bottom. Water temperature ranged from 1.5–3 C during the winter sampling periods.

Aquarium Studies

The large number and small sizes of juvenile spring-run chinook salmon made it difficult to identify many individual branded salmon in the field. Hence, during the fall of 1965 a stream aquarium (Fig. 2) was constructed at the University of Idaho for work with juvenile spring-run chinook salmon.

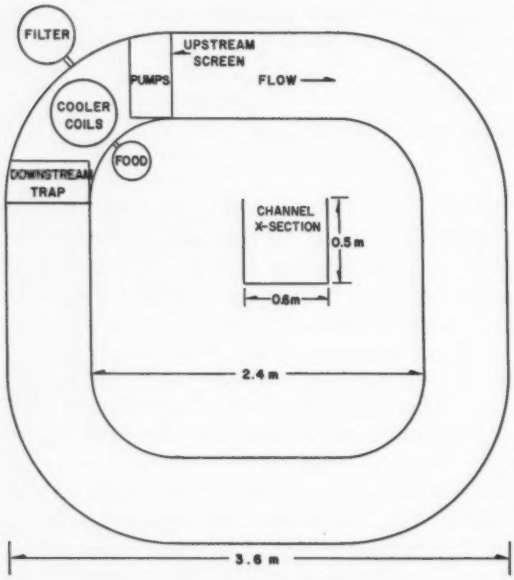

FIG. 2. Stream aquarium, with inside walls of glass. Perforated zigzag pipe buried in substrate was used to supply food through aquarium.

The aquarium, constructed of plyboard, was 0.6 m wide, 0.5 m high, and 12 m long, and had a glass inside wall. A black polyethylene sheet (with viewing slits) covered the aquarium. Incandescent lights were turned on for a 13-hr day (0800–2100 Pacific Standard Time). A mechanical device increased the light intensity, measured at the water surface, from 0 to 43 lumens/m^2 (0–40 ft-c) within 1 hr each morning and reduced the light to darkness each evening.

Brine shrimp (*Artemia salina*) were forced through a perforated pipe laid in the gravel of the aquarium bottom to simulate emergence and drift of aquatic insects. Some food was in the aquarium all day, but there were two periods of greatest abundance, at about 0800–0900 and 1700–1800 hr.

An upstream screen barrier and downstream trap were installed in one corner of the aquarium. Fish could leave the aquarium by entering the downstream trap, and those which did were discarded.

Water in the aquarium was circulated by two ⅓-hp pumps. Water temperature was maintained at about 12 C with the aid of cooler coils present between the barriers.

Gravel 5 cm in largest diam was arranged in the aquarium to provide fast and slow current areas. There were three pools 30 cm deep, alternated with two shallow areas 7 cm deep. In one 1.2-m section the water was 15 cm deep near the inside glass and 5 cm deep next to the plyboard. We arbitrarily divided the bottom into 0.3-m gridded reference sections starting at the upstream barrier.

On April 28, 1966, 30 spring-run chinook salmon (34–39 mm) from a hatchery on Rapid River near Riggins, Idaho, were released in the aquarium. We identified fish by parr-mark arrangements combined with adipose fin removal and/or caudal fin notches. Positions of all fish were recorded from May 9 to 23, 1966 at 0830, 1300, and 2000 hr. After occasional observations in midmorning and midafternoon we were willing to accept thrice-daily sightings as representing movements fairly. The individual positions of each fish were plotted and the most frequently occupied 0.11-m^2 area was termed the home station. Movements were then treated as distances away from the home station. To learn if the fish moved longer distances in the morning than in the afternoom we measured the observed morning–noon, noon–evening, and morning–evening movements. Fish could move about 10 m from one end of the aquarium to the other, but fish in the middle of the aquarium could move only 5 m up- or downstream. There were 21 fish in the aquarium during the first week and 10 for the second. Work was confined to May because of fish availability and work schedules in the aquarium.

RESULTS

Within-Day Movements in Field Study

Of 151 movements of steelhead observed in Johnson Creek during July and August 1965, and in July 1966, 86% were less than 6 m (Fig. 3). The frequency distributions of distances moved from morning to noon, noon to evening, and morning to evening were similar.

Movements over Two or More Days in Field Studies

Of 59 movements of steelhead between sequential sightings 1 day apart, 68% were less than 6 m, with 53% being less than 3 m (Table I). Of 56 sequential sightings from 10 to 14 days apart, 63% were less than 6 m.

Observation dives were made outside of both study sites, but branded fish were never seen more than 9 m from the study areas. On days when three or more observation dives were made in the Lochsa River and Johnson Creek study sites, an average of 60% of all steelhead juveniles originally branded were seen (Table II).

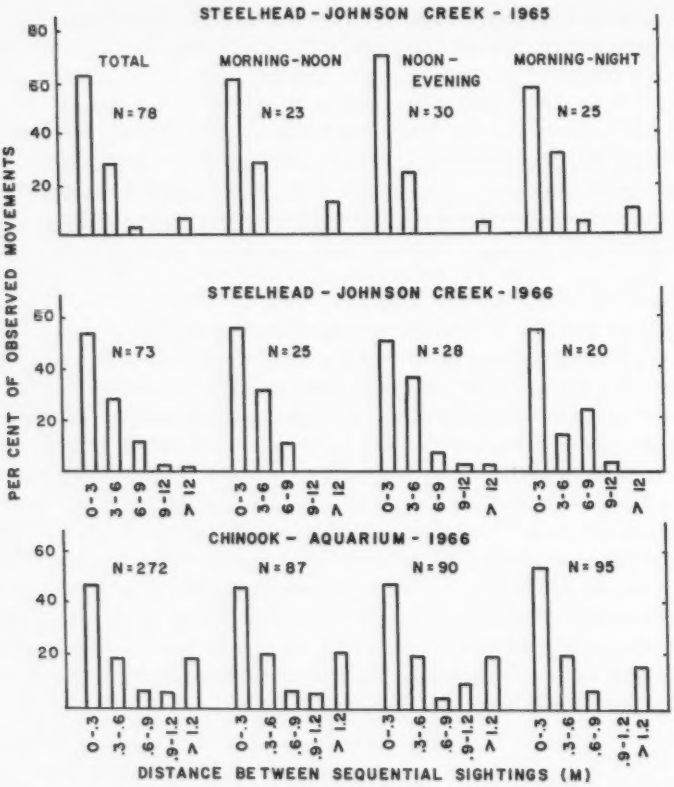

FIG. 3. Observed movements of branded juvenile steelhead and chinooks. Movements are distances between positions at which fish were sighted in the same day.

DAYTIME MOVEMENTS IN AQUARIUM STUDIES

Of 290 movements of chinook juveniles from a home station in the stream aquarium (defined as the most frequently occupied 0.11 m² area) 65% were less than 0.6 m. The data, obtained May 9–23, 1966, were as follows:

Distance moved (*m*)	.0–.3	.31–.6	.61–.9	.91–1.2	>1.2
Per cent of observed movements	45	20	5	4	26

NIGHT LOCATIONS

At night in summer, both species were inactive and usually occupied positions different from day stations. The night positions of steelhead trout were found near the shoreline out of the main flow (Fig. 4, for example). Steelhead were found in depressions in sand, under rocks, between rocks, on

TABLE 1. Distance branded steelhead trout moved between sequential sightings on successive days and over longer periods in the Lochsa River in 1965 and Johnson Creek in 1965 and 1966.

Interval	Distance moved (*m*)				
	0–3	3–6	6–9	9–12	>12
Lochsa River, July 29–Aug. 23, 1965					
July 29–Aug. 12 (14 days)	1	2	0	0	0
Aug. 12–13 (1 day)	12	1	1	1	0
Aug. 12, 13–23 (10–11 days)	9	1	2	0	0
Johnson Creek, Aug. 4–19, 1965					
Aug. 4–18 (14 days)	5	2	2	0	1
Aug. 18–19 (1 day)	5	1	1	1	2
Johnson Creek, July 7–21, 1966					
July 7–8 (1 day)	6	3	3	1	2
" 8–19 (11 days)	4	3	3	1	3
" 20–21 (1 day)	8	4	4	3	0
" 7–21 (14 days)	6	2	4	2	3
Per cent of 59 sequential sightings on successive days	53	15	15	10	7
Per cent of 56 sequential sightings 10–14 days apart	45	18	20	5	12

sandy bottoms of pools, and near edges of shallow riffles. All branded steelhead that we observed moved to a different habitat at night. The distance between day and night sightings for given fish varied from less than 1 m to as much as 45 m. Very rarely were steelhead seen off the stream bottom in current at night. We observed two fish in the same night locations on different nights.

Chinook salmon behaved somewhat differently from steelhead trout at night. They were farther inshore, sometimes in water less than 5 cm deep. They were found on sandy bottoms and in surface waters in the same pool on the same night. During the day they were found in these same waters at all depths. Aquarium observations with an infrared light and viewer suggested that the chinook salmon settled to the bottom after dark. During one period in January 1966, when the aquarium was in a testing phase, a very dim glow was present in the incandescent light bulbs. During these nights more chinook appeared in the surface waters in predawn observations than on nights when the lights were off.

WINTER LOCATIONS

The fish we located in winter by using "primacord" were up to 15 cm deep in the substrate. During the winter of 1965–66 four branded steelhead (90–98 mm, age I) were found in the bottom on the same home area they

TABLE II. Percentages of branded steelhead trout re-observed on dives in study areas in the Lochsa River (1965) and in Johnson Creek (1965 and 1966). A dive was an observation period and lasted about 1 hr.

Date branded fish released (no. released)	Date fish observed	No. of dives in that day	% of released fish observed
	Lochsa River, 1965		
July 26 (37)			
July 29 (12)			
	Aug. 13	3	70
	" 23	1	38
	" 31	1	20
	Johnson Creek, 1965		
July 20 (42)			
	Aug. 4	3	50
	" 17	1	26
	" 18	3	48
	" 19	3	43
	Johnson Creek, 1966		
June 28 (31)	July 7	4	71
	" 8	3	68
	" 19	3	68
	" 20	3	65
	" 21	3	71
	" 26	1	35
	" 27	1	45

occupied the previous summer. Two of the branded fish were located in the middle of the stream and the others were found near the shore off the main current. Juvenile steelhead trout from age-classes I and II and numerous unbranded 0-age chinook salmon were found in the substrate during winter sampling. Occasionally fish were found on the bottom rather than in the substrate. During June 1966, two branded fish were caught within several meters of the home area they had occupied the previous summer. Both fish remained there during the summer of 1966.

DISCUSSION

We conclude that juvenile steelhead trout in Johnson Creek and the Lochsa River often maintain a permanent station during the day in the summer months. Some movement occurs, probably for feeding or defense of territory, but in nearly all cases the fish return to a specific station after such movements. Most of the diurnal movements we observed were less than 6 m, with about 60% being less than 3 m. In brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) (Kalleberg, 1958) and in coho salmon (*Oncorhynchus kisutch*) (Mason, MS, 1966) feeding is usually performed from a particular spot. In aquarium studies we found a strong permanence of station in spring chinook salmon.

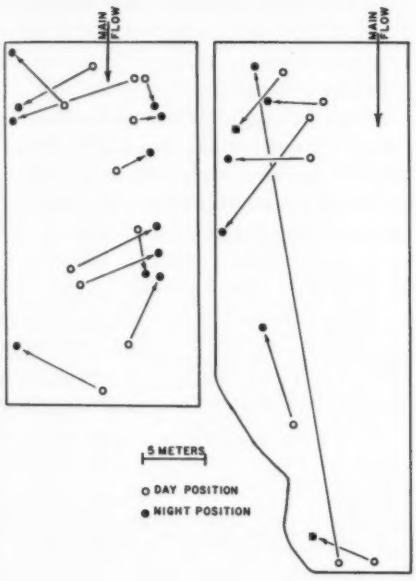

FIG. 4. Day and night locations of branded juvenile steelhead trout in Johnson Creek study site, August 25–27, 1966 (*left*), and the Lochsa River study site, August 13, 1965 (*right*).

Our study has also shown that some steelhead trout maintain a specific home area during the summer. Brook trout (*Salvelinus fontinalis*) (Shetter, 1937), brown trout (Schuck, 1943), cutthroat trout (Miller, 1957), and Atlantic salmon (Saunders and Gee, 1964) all are reported to remain in a home area during some or all life phases. Gerking (1959) lists other species demonstrating restricted movements.

Density of populations of steelhead and chinook salmon could be expected to have an effect on movements. We found in other work with chinook in our aquarium that excessive fish densities became adjusted by emigration. We assumed in both field and laboratory studies that densities were not excessive. Another necessary assumption was that the habitats studied were adequately stocked or seeded with young fish.

Juvenile steelhead trout and chinook salmon usually occupied positions at night that were different from day stations. Hoar (1953) reported steelhead trout moved into shallow water, settled to the bottom, and remained inactive at night. Most of the steelhead trout and chinook salmon we observed at night had moved inshore from their day stations to quieter, shallower waters.

Like steelhead trout and some chinook salmon, the following salmon species have been reported to be inactive at night: coho salmon (Hoar, 1953; Chapman, 1962; Mason, MS, 1966), Atlantic salmon (Hoar, 1953), kamloops trout (*Salmo gairdneri*) (Stringer and Hoar, 1955), brown trout (Lindroth, 1955; Swift, 1962, 1964), and rainbow trout (Newman, 1956). We also noted that cutthroat trout and Dolly Varden were inactive on the bottom at night.

Winter locations of steelhead trout and chinook salmon were primarily under or between rubble particles and young fish were seen rarely without diligently searching in the substrate.

Hartman (1963, 1965) found that juvenile steelhead trout "hide" during winter. He suggested the response protected the fish against possible predation, downstream displacement, and possible ice scouring. Young sea trout, *Salmo trutta* (Lindroth, 1955), and Atlantic salmon (Saunders and Gee, 1964; Allen, 1941) have been reported to be inactive or in the substrate in winter, with *S. salar* being found in the same areas occupied the previous summer.

We found no branded chinook salmon in the study areas in winter but numerous unbranded chinook salmon were present. On Johnson Creek, two branded steelhead trout were observed in summer in the same location at times nearly 1 year apart. It is possible that steelhead spend a year or more in one home area. Some brook trout (Shetter, 1937) and brown trout have been reported by Shetter (1937) and Schuck (1943) to occupy the same place for two consecutive summers.

The factors causing station permanence are not obvious. We like Hoar's (1966) general description of behavior as "a limited number of innate, stereotyped patterns of movement which are released by specific stimuli and guided or oriented by definite features of the environment of the animal." Hoar relegates stimuli to the environment.

Environmental features likely to act as stimuli releasing motor patterns leading to fixation to a station or territory in an individual young chinook salmon or steelhead are: water velocity and depth; substrate character; possibly cover in the form of overhead turbulence or stationary objects; and density of conspecifics or other fishes.

MacKinnon and Hoar (1953), Hoar (1954), Lindroth (1955), Kalleberg (1958), and Saunders and Gee (1964) suggest that positive reaction to water velocity aids the fish in maintaining a position in the stream. The positive rheotactic behavior displayed by steelhead trout and chinook salmon would obviously be necessary in maintaining permanent positions. Lindroth (1955) found that fish maintaining territories in moving water tended to move away from these territories in still water.

Ability to adapt quickly to low light intensities (Ali, 1959) and movement to the bottom at night could also help fish to remain in stable positions (Hoar, 1953; Stringer and Hoar, 1955; Chapman, 1962). We know that young steelhead move to or into the substrate at night, and sometimes take the same night position on successive nights at locations quite different from day stations. We now wonder if night cover needs may bear upon the environmental stimuli which lead to habitat selection and station locations.

Wynne-Edwards (1962) and Ardrey (1966), among others, have discussed at length the importance of territoriality and permanence of station and home area. There appear to be definite benefits accruing to fish demonstrating such behavior. In stream-dwelling salmonids some obvious possible benefits include: (1) familiarity with cover; (2) the psychological advantage enjoyed by territory

holders during agonistic encounters; (3) assurance of sufficient space to guarantee access to food supplies; and (4) maintenance of distances from nearest neighbors sufficient to prevent excessive pathogen transfer.

We know that juvenile chinook salmon and steelhead trout usually have a fixation to a very limited area. We speculate that as growth occurs and stimuli such as water depth and velocity, temperature, food supply, or social relationships no longer release holding behavior, the fish move to another area and that the new area provides the stimuli releasing motor patterns that, in turn, cause fixation to a station or limited area.

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Effect of Metopirone on Pituitary–Interrenal Function in Two Teleosts, Sockeye Salmon (*Oncorhynchus nerka*) and Rainbow Trout (*Salmo gairdneri*)[1]

By Ulf H. M. Fagerlund, J. R. McBride, and Edward M. Donaldson

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

ABSTRACT

Metopirone (SU 4885), an inhibitor of 11β-hydroxylation of adrenocorticosteroids, was administered intramuscularly to adult castrated sockeye salmon (*Oncorhynchus nerka*) and intact rainbow trout (*Salmo gairdneri*). Marked hypertrophy of interrenal cells pointed to an increase in the activity of the interrenal tissue of both species. Increased cortisol concentrations in trout at low dosage levels of metopirone suggested that the drug was stressful. However, decreased concentrations at higher dosage levels in trout, and decreased cortisol concentrations in all salmon indicated that the drug was also exerting a marked inhibitory effect on 11β-hydroxylation. In salmon, a repository ACTH preparation (acthar) brought about interrenal hypertrophy similar to that produced by metopirone. Metopirone also induced degranulation and hyperplasia of the cells of the palisade-like layer in the rostral region of the pars distalis in both species of fish. These cells were the only cell type of the pars distalis that were stained with lead-haematoxylin. The evidence indicates that they are the corticotrops.

INTRODUCTION

The marked physiological and morphological changes that occur in the various organs and tissues of Pacific salmon at the time of sexual maturation have been shown to be similar to those associated with hyperadrenocorticism in man (Robertson and Wexler, 1959, 1960). Although these conclusions were supported by high corticosteroid concentrations in maturing and mature salmon (Robertson and Wexler, 1960; Idler et al., 1959; Schmidt and Idler, 1962), Fagerlund (1967) noted that cortisol concentrations remained low if the fish were not stressed. The elucidation of the role of the pituitary–interrenal system in the changes taking place in the Pacific salmon has been hampered by the failure to identify corticotropin-producing cells in the pituitaries of these fish, although marked changes in pituitary activity, per se, have clearly been demonstrated (Robertson and Wexler, 1962b; van Overbeeke and McBride, 1967).

Liddle et al. (1959) found that metopirone inhibits 11β-hydroxylation of adrenal steroids and, via a negative-feedback mechanism, stimulates corticotropin release. In lower vertebrates, metopirone has been used to study pituitary–adrenal function in eel (Olivereau, 1963, 1965; Ball and Olivereau, 1966; Olivereau and Ball, 1963b), *Poecilia latipinna* (Ball and Slicher, 1962; Olivereau and Ball, 1963a; Ball and Olivereau, 1966), *P. formosa* (Olivereau

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and Ball, 1963a), African lungfish (Janssens et al., 1965), and common frog (van Kemenade and van Dongen, 1965). In the present study, metopirone was used in an attempt to identify the corticotrops of sockeye salmon (*Oncorhynchus nerka*) and rainbow trout (*Salmo gairdneri*). Pituitary sections were stained with MacConaill's (1947) lead–haematoxylin. In the rostral pars distalis this stain is specific for the corticotropic cells which has been demonstrated in lower vertebrates by Olivereau (1965, 1967) and Ball and Olivereau (1966). The effect of metopirone on interrenal tissue and plasma cortisol concentrations in the two salmonids were correlated with the changes seen in the pituitaries, and, in the case of salmon, with the effect of a repository adrenocorticotropin (ACTH) preparation.

MATERIALS AND METHODS

Oncorhynchus nerka

Sexually immature adult sockeye salmon, weighing between 1450–2760 g, were caught when entering Great Central Lake on Vancouver Island, British Columbia, on the migration route to their spawning grounds, and were transferred to 1.8-m circular aerated aquaria. The method of capture and transport to the Vancouver Laboratory and the procedures used in castration have been described (McBride et al., 1963). The fish, while in captivity, were maintained under natural photoperiod conditions. The gonads, at the time of castration (July 1966), were immature. Some 3 to 4 weeks after gonadectomy, the fish were induced to feed voluntarily (McBride, 1967). Experiments commenced 6 months after gonadectomy (January 1967).

In one group of fish, metopirone (SU 4885, Ciba Co. Ltd., Dorval, Quebec) dissolved in peanut oil (100 mg/ml) was administrated intramuscularly to castrated female fish, once a day. The dose varied from 13.3 to 20.8 mg per 1000 g of fish. Initially, doses of 35–45 mg were tried, but the fish so treated did not survive beyond 24 hr. Controls received 0.33 ml of peanut oil, regardless of body weight.

In a second group of female castrates, repository, porcine ACTH in 16% gelatin (acthar, Amour Pharmaceutical Co., Kankakee, Ill., 20 IU/ml) was administrated in daily intramuscular injections of 8.0–13.6 IU per 1000 g of fish. Controls receive 1 ml of 0.6% saline, regardless of body weight.

To obtain blood, salmon were rapidly anaesthetized in 2-phenoxyethanol and, in order to minimize the effect of the stress involved on cortisol concentrations (Fagerlund, 1967), blood was drawn immediately from the caudal artery with heparinized vacutainers. It was centrifuged at 2 C and 3000 *g*, and the plasma stored at –25 C until used. Cortisol was determined fluorimetrically in single 1-ml plasma samples, as previously reported (Donaldson et al., 1968).

Salmo gairdneri

Rainbow trout were obtained from Sun Valley Trout Farm, Port Coquitlam, British Columbia. They were of mixed sex, and had an average weight of 250 g. The fish were held at 12 C in 160-litre, self-cleaning, fiber glass aquaria equipped with an aerator (Donaldson and McBride, 1967).

Zero control trout received no injections. Two peanut oil control groups received three or five daily intraperitoneal injections of 0.5 ml of peanut oil. Five groups received three intraperitoneal injections of 25, 50, or 100 mg of metopirone in 0.5 ml peanut oil, or five injections of 25 or 50 mg of metopirone. Blood was sampled 4 hr after the last injection, only one blood sample being taken from each fish.

Prior to blood sampling, trout were anaesthetized without disturbance by pouring a concentrated solution of tricaine methanesulphonate into the aquarium (Donaldson and McBride, 1967). The fish were removed 10 min later, and blood samples were collected from the dorsal aorta in the tail region, using 4-ml heparinized vacutainers and 21 G holder needles (Becton, Dickinson & Co., Canada, Ltd.). The blood was centrifuged in the vacutainers at 2 C and 3000 *g* for 10 min, and the plasma stored at −25 C.

Histological Techniques

Immediately after the collection of the blood sample, the spinal cord was severed, just caudal to the head. The pituitary gland and the interrenal tissue of the fish were excised, fixed in Bouin–Hollande-sublimate, and embedded as described previously (van Overbeeke and McBride, 1967). Serial longitudinal sections of the pituitary were cut at a thickness of 5 μ and stained by the following techniques: Masson's trichrome, including ponceau de Xylidine (Culling, 1957), periodic acid-Shiff (PAS)-orange G (Culling, 1957), and lead–haematoxylin (MacConaill, 1947). Sections of the interrenal tissue were cut transversely at a thickness of 5 μ and stained with either haematoxylin–eosin or Masson's trichrome.

Mean nuclear diameters of the interrenal cells were obtained by measuring the diameters of 10 nuclei selected at random from mainly one cluster of cells from the interrenal tissue of each fish. Several other clusters in each fish were examined in order to establish whether nuclear diameters varied from cluster to cluster. Such variation was not found.

In preliminary experiments, metopirone was administrated to maturing intact sockeye and coho salmon (U. H. M. Fagerlund and J. R. McBride, unpublished observations). In these fish, the interrenal cells and cell nuclei vary considerably in size, with clusters of cells showing quite different levels of activity, which made it difficult to detect changes without including the whole adrenal in the measurements. After castration, the interrenal cells become quiescent, and the interrenal tissue appears uniform. Castrated fish were, for this reason, chosen for the present experiments.

A number of castrated salmon from the group of fish used for this study, held under identical conditions but not receiving injections, were killed at the time of the present experiment for a different study. The interrenal tissue of these fish (5 ♂, 5 ♀) was quiescent. The mean and SD of nuclear diameters were 6.40 $\pm$ 0.28 μ and 6.44 $\pm$ 0.35 μ respectively.

RESULTS

Plasma Cortisol Concentrations

Oncorhynchus nerka. — The effect of injections of peanut oil and metopirone in peanut oil on cortisol concentrations in plasma of gonadectomized female sockeye salmon are shown in Table I. At the time of the 10th injection,

Table I. Effect of peanut oil and metopirone injections on cortisol concentrations (μg/100 ml) in plasma of castrated female sockeye salmon.

Vehicle	Drug	Dose per kg body wt (mg)	Cortisol concentration: 0 injection	3–5 injections		10 injections		Remarks
			Resting	Resting	Stressed	Resting	Stressed	
Peanut oil	–	–	0.7		26.6	18.2	42.3	
" "	–	–	1.5		24.8			Died after the 5th injection
" "	Metopirone	13.3	2.5	4.7	7.2			Killed after the 4th injection
" "	"	13.7	0.9		10.1			Died after the 3rd injection
" "	"	16.7	0.9		4.2	15.5	18.2	
" "	"	20.8	1.5		3.7	10.5	10.0	

both treatments had caused increases in cortisol concentrations. In order to assess the effectiveness of metopirone in inhibiting steroid 11β-hydroxylation during acute stress, blood samples were taken 4 hr after the fish had been subjected to the stress of a previous blood sampling and injection. At the time of the 10th injection, the acute stress produced only a minor increase in cortisol concentration in fish receiving metopirone, whereas in a peanut oil-injected fish, the concentration increased from 18.2 to 42.3 μg/100 ml. Cortisol concentrations in samples taken after acute stress from fish which had received three to five injections were considerably higher in the peanut oil controls than in metopirone-injected fish.

Cortisol concentrations in plasma of gonadectomized male and female sockeye salmon which had received injections of long-acting acthar gel are shown in Table II. Plasma samples were in some cases taken when fish were resting and, from all fish, 4 hr after the fish had been subjected to the acute stress of a previous blood sampling and injection. Comparison of the cortisol concentrations at the time of the initial daily injection (resting values) with those taken 4 hr later (stress values) indicates that the cortisol concentration was maintained at the maximal level throughout the duration of the experiment.

TABLE II. Cortisol concentration (μg/100 ml) in plasma of castrated male and female sockeye salmon injected with acthar.

Sex	Vehicle	Drug	Dose per kg body wt (IU)	Cortisol concentrations				
				0 injection	3–4 injections		10 injections	
				Resting	Resting	Stressed	Resting	Stressed
Male	Saline	–	–			20.0		
"	Gel	ACTH	9.2	1.1		45.0	59.0	59.0
"	Gel	ACTH	8.6	0.7		39.5	54.8	42.1
Female	Saline	–	–			31.6		
"	Gel	ACTH	8.0	1.9		83.0	102.0	104.0
"	"	"	13.8	10.8	83.1	83.0		
"	"	"	13.6	1.9	140.0	172.5		

Salmo gairdneri. — The mean plasma cortisol concentrations in zero time controls and trout injected with peanut oil and metopirone in peanut oil are presented in Fig. 21. The mean cortisol concentration in fish which received three injections of 25 mg/kg metopirone was higher than that in the zero time control fish, or the group which received three injections of peanut oil. The mean plasma cortisol concentration in the groups receiving three injections of 100 mg/kg was significantly lower than the mean plasma cortisol concentration in the 50-mg/kg group ($p < 0.01$) and the 25-mg/kg group ($p < 0.001$). The mean plasma cortisol concentration in fish which had received five injections of peanut oil was 4.35 μg/100 ml. There was a progressive decrease in mean plasma cortisol concentration to 3.45 μg/100 ml in the 25-mg/kg group for 5 days, and to 2.85 μg/100 ml in the 50-mg/kg groug. The correlation coefficient for cortisol concentrations in fish receiving three injections of 25, 50, and 100 mg of metopirone was −0.83.

INTERRENAL TISSUE

Oncorhynchus nerka. — Mean nuclear diameters of the adrenocortical cells of castrated female sockeye salmon which had received intramuscular injections of 0.6% saline, acthar gel, peanut oil, and metopirone in peanut oil are presented in Table III. The nuclear diameters in any one fish were quite uniform in size (Fig. 1 and 2). Mean nuclear diameters were smallest in two fish injected with saline (6.45 and 6.48 μ). The range of mean nuclear diameters in five fish injected with acthar was 7.87–8.08 (Fig. 3 and 4). One fish injected with peanut oil had a slightly larger mean nuclear diameter than the saline controls (Fig. 5 and 6). The mean nuclear diameters (range 7.91– 8.42) of four fish which had received metopirone were similar to those injected with acthar (Fig. 7 and 8). Both acthar- and metopirone-treated fish showed prominent nucleoli. The data do not indicate a difference between male and female fish, or between those receiving 5 and those receiving 10 injections of either drug.

TABLE III. Effect of acthar and metopirone on the nuclear diameters (mean ± SD) of interrenal cells of adult castrated sockeye salmon.

Sex	No. of injections	Vehicle	Drug	Dose per kg body wt	Mean nuclear diameter ±SD	Treatment mean ±SD
Male	5	Saline	–	–	6.45±0.23	6.47
Female	5	"	–	–	6.48±0.39	
"	5	"	ACTH	13.6 IU	8.02±0.11	7.99±0.5
"	5	"	"	13.8 "	8.02±0.11	
"	5	"	"	13.8 "	7.93±0.19	
"	10	"	"	8.0 "	8.05±0.11	
Male	10	"	"	9.2 "	7.87±0.21	
Male	10	"	"	8.6 "	8.08±0.21	
Female	10	Peanut oil	–	–	7.02±0.22	7.02
"	5	" "	Metopirone	13.3 mg	7.91±0.22	8.16±0.29
"	10	" "	"	13.7 "	8.41±0.32	
"	10	" "	"	16.7 "	8.42±0.38	
"	10	" "	"	20.8 "	7.91±0.15	

Salmo gairdneri. — The mean interrenal nuclear diameters of intact trout and trout which had received three or five daily injections of peanut oil or metopirone in peanut oil are presented in Fig. 22. The trout injected with peanut oil had greater interrenal nuclear diameters than those which had received no injections. The trout injected with metopirone, in addition to larger mean interrenal nuclear diameters, exhibited more prominent nucleoli than those injected with peanut oil (Fig. 9–12), the increase in nuclear size being related to the dose and the number of injections. The mean interrenal nuclear diameter of the fish receiving five injections of metopirone, 50 mg/kg, was significantly higher than that of the control fish receiving five injections of peanut oil ($p < 0.02$). The correlation coefficient for mean nuclear diameters of fish receiving 25, 50, and 100 mg of metopirone was 0.85.

PITUITARY GLAND

The pars distalis of Pacific salmon and of rainbow trout consists of two distinct regions; the rostral area and the much larger proximal area (Robertson and Wexler, 1962a, b; van Overbeeke and McBride, 1967). Delineation of the rostral region is simplified first by extensions of the neurophysis between this region and the proximal zone, and also by the presence of a palisade-like layer of cells located at the ventromedial edge of the rostral zone (Fig. 13). In midsaggital sections, the palisade-like layer of cells of control fish of either species seldom exceeded three to four cells in depth; the cell nuclei were mainly circular in shape with inconspicuous nucleoli, and the cytoplasm was packed with fine lead–haematoxylin-positive granules (Fig. 14 and 19). The cytoplasm of the remaining cells of the rostral area, as well as those of the proximal region,

Explanation of Figures

FIG. 1. Interrenal tissue of a sockeye salmon treated with 0.6% saline for 10 days. Interrenal cells border on a branch of a cardinal vein (V). Masson trichrome. ×125.

FIG. 2. Higher magnification of interrenal cells shown in Fig. 1. Cell nuclei uniform in size and nucleoli inconspicuous. Masson trichrome. ×500.

FIG. 3. Interrenal tissue of a sockeye salmon injected with acthar (a total of 10 daily injections, 8.0 IU per kg of fish per day). Contrast with Fig. 1. Masson trichrome. ×125.

FIG. 4. Higher magnification of the interrenal cells shown in Fig. 3. Cells hypertrophied; also note prominence of nucleoli. Cytoplasm appears to be vacuolated. Masson trichrome. ×500.

FIG. 5. Interrenal tissue of a sockeye salmon treated with peanut oil for 10 days. Masson trichrome. ×125.

FIG. 6. Higher magnification of interrenal cells shown in Fig. 5. Masson trichrome. ×500.

FIG. 7. Interrenal tissue of a sockeye salmon treated with SU 4885 for 10 days (16.7 mg per kg of fish per day). Cells exhibit marked hypertrophy. Contrast with Fig. 5. Masson trichrome. ×125.

FIG. 8. Higher magnification of interrenal cells shown in Fig. 7. Cortical nuclei are hypertrophied and show prominent nucleoli. Contrast with Fig. 4 and 6. Masson trichrome. ×500.

FIG. 9. Interrenal tissue of a trout treated with peanut oil for 3 days. Nuclei uniform in size with nucleoli rarely discernible. Masson trichrome. ×500.

FIG. 10. Interrenal tissue of a trout (not included in Fig. 21 and 22) treated with SU 4885 for 5 days (5.5 mg per kg of fish per days). Nucleoli prominent in a few of the cells. Masson trichrome. ×500.

FIG. 11. Interrenal tissue of a trout treated with SU 4885 for 5 days (25 mg per kg of fish per day). Note marked hypertrophy of cortical tissue. Nucleoli prominent in a few cells. Contrast with Fig. 9 and 10. Masson trichrome. ×500.

FIG. 12. Interrenal tissue of a trout treated with SU 4885 for 3 days (100 mg per kg of fish per day). Cortical tissue markedly hypertrophic, nuclei visibly enlarged, and nucleoli prominent. Masson trichrome. ×500.

FIG. 13. A midsaggital section of the pituitary of a saline-treated sockeye salmon showing the proximity of the neurohypophysis (N) to a portion of the rostral area (R) of the pars distalis. Separating the lead-haematoxylin-negative follicular cells of the rostral area from the neurohypophysis is a palisading layer of lead-haematoxylin-positive cells (arrows). Lead-haematoxylin. ×125.

FIG. 14. Higher magnification of the lead-haematoxylin-positive cells shown in Fig. 13. Note presence of lead-haematoxylin-negative follicular cells (F). Lead-haematoxylin. ×500.

FIG. 15. The palisading layer of lead-haematoxylin-positive cells of the pituitary (rostral area) of a salmon treated with acthar for 10 days. Lead-haematoxylin. ×125.

FIG. 16. Higher magnification of the palisade layer shown in Fig. 15. A ramification of the neurohypophysis (N) is shown separating two layers of lead-haematoxylin-positive cells. The cytoplasm of the lead-haematoxylin-positive cells is packed with fine dense granules. Lead-haematoxylin. ×500.

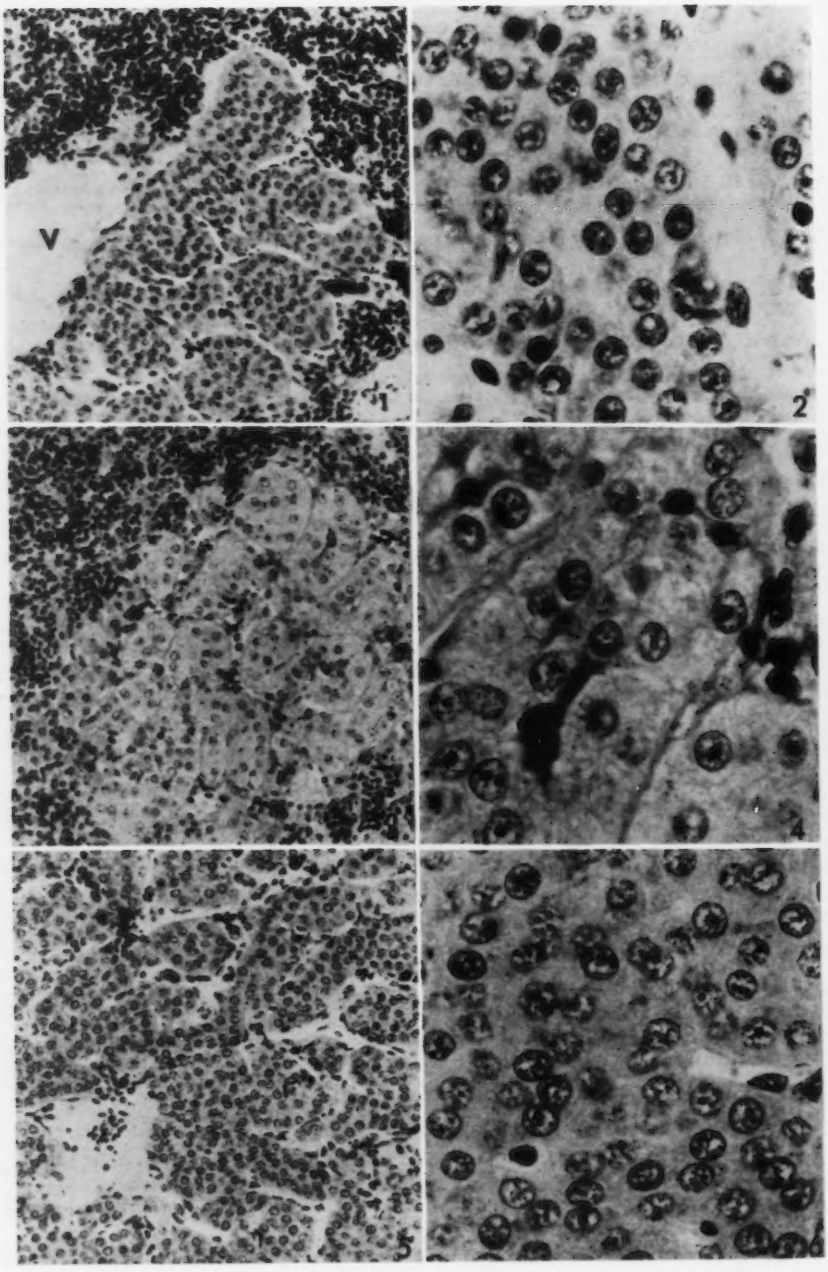
FIG. 17. A portion of the rostral region (midsaggital section) of the pituitary of a sockeye salmon treated with SU 4885 for 10 days. Section counterstained with erythrosine to facilitate differentiation between the neurohypophysis (N), follicles (F), and the palisade layer (arrows). Note the virtual complete absence of lead-haeatoxylin-positive granules in the palisade layer. Contrast with Fig. 13 and 15. Lead-haematoxylin–erythrosine. ×125.

FIG. 18. Higher magnification of the cells of the palisade layer shown in Fig. 17. Contrast with Fig. 14 and 16. Lead-haematoxylin. ×500.

FIG. 19. Portion of the pituitary (rostral region of the pars distalis) of a trout treated with peanut oil for 5 days. The palisade layer of lead-haematoxylin-positive cells (arrows) separates the lead-haematoxylin-negative follicle cells (F) from the neurohypophysis (N). Lead-haematoxylin. ×125.

FIG. 20. Pituitary of a trout treated with SU 4885 for 3 days. Note virtually complete absence of lead-haematoxylin-positive granules in the cells of the palisade column (arrows). Contrast with Fig. 19. Lead-haematoxylin. ×125.

(FIG. 1–20 *follow*)



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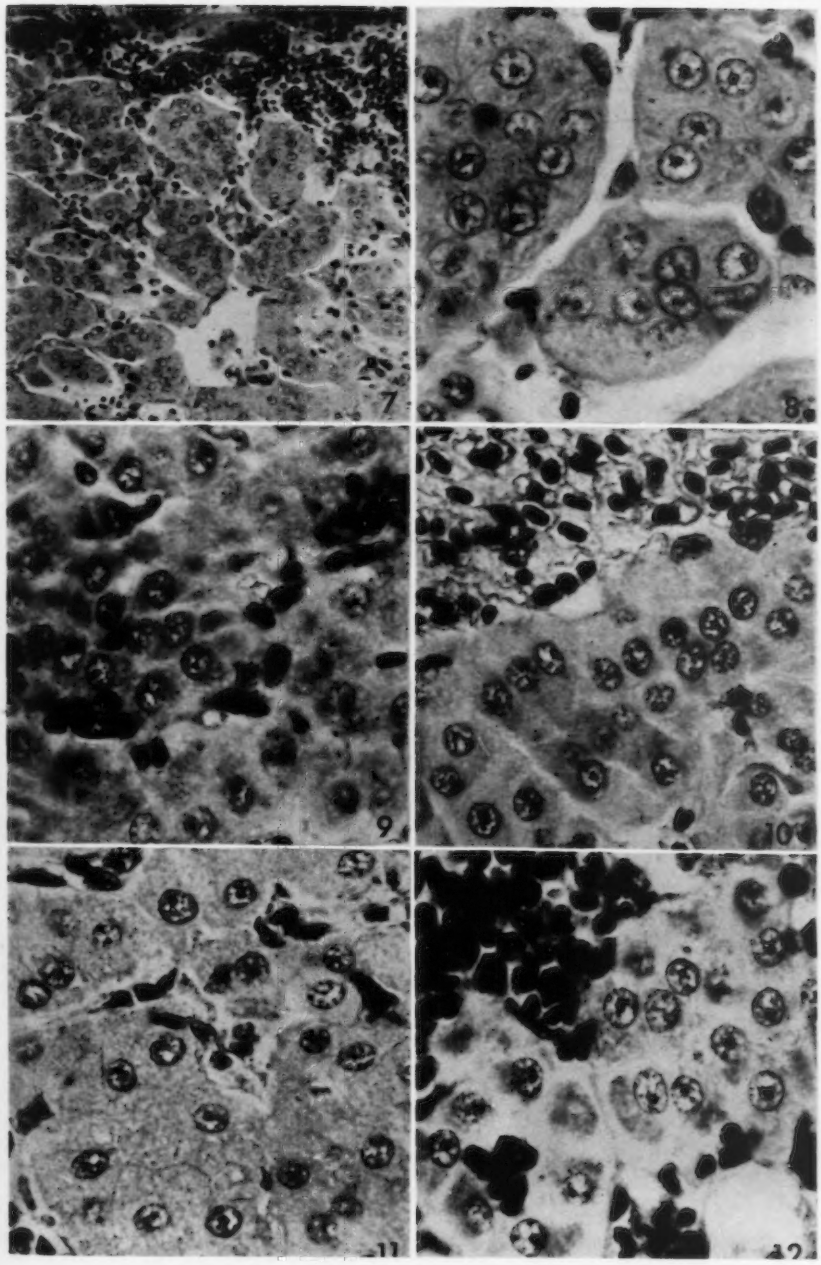

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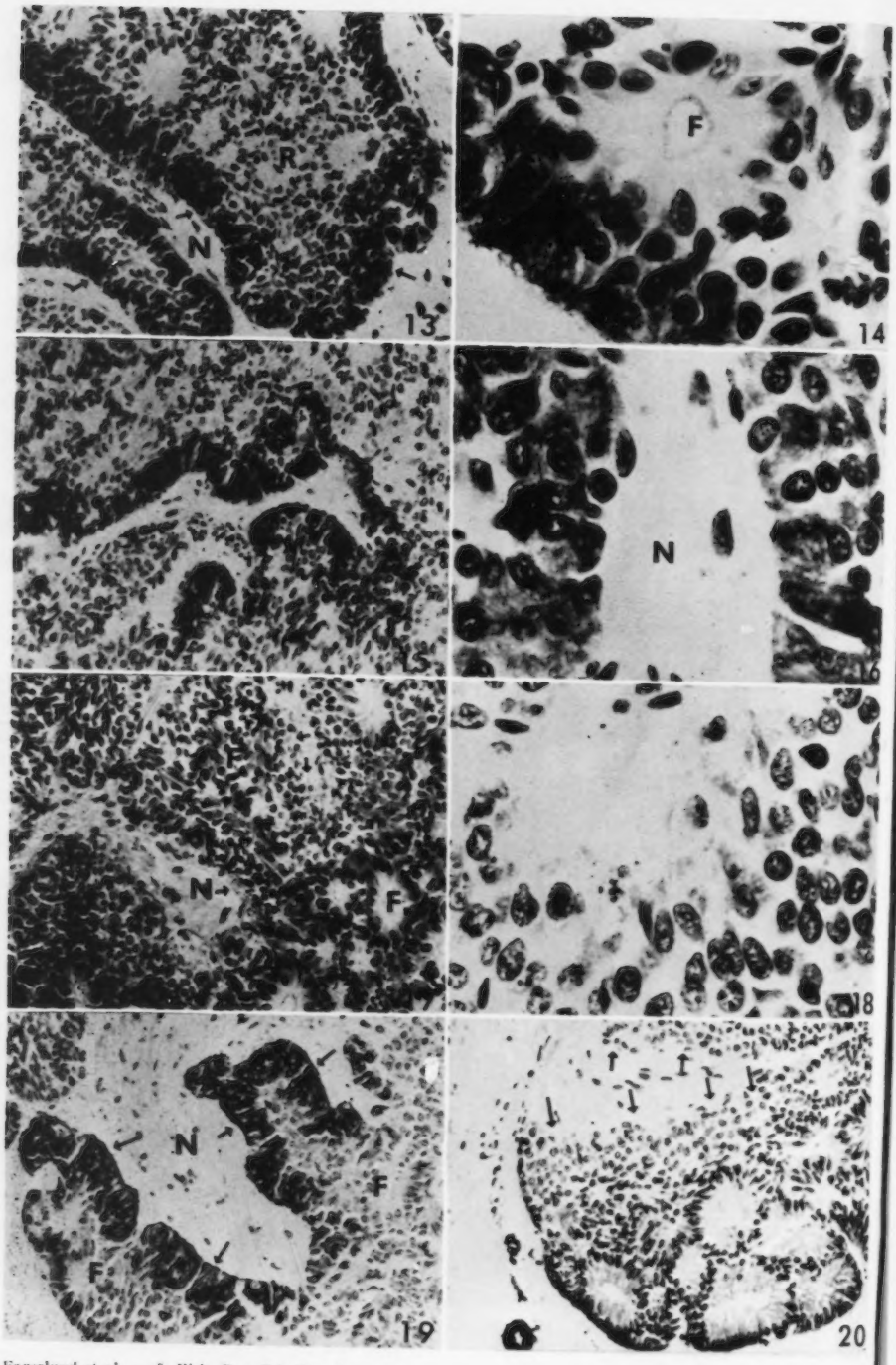

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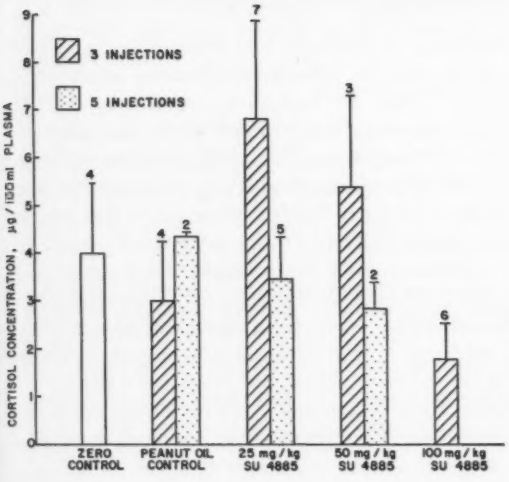

FIG. 21. Effect of metopirone on plasma cortisol concentrations (mean + SD) in rainbow trout. Numbers of fish are indicated by figures above the bars. Correlation coefficient for values of fish receiving three injections of 25, 50, and 100 mg of SU 4885: $r = -0.83$.

were lead–haematoxylin negative. Also stained by lead–haematoxylin were the cytoplasmic granules in the cells of the pars intermedia.

Metopirone treatment was characterized in both trout and salmon by a degranulation of the lead–haematoxylin-positive cells of the pars distalis (Fig. 18 and 20). Furthermore, at the higher dosages used in the trout and in all the salmon, the palisade-like layer was visibly thickened, often exceeding 8–10 cells in depth (Fig. 20). None of the other cell types present in the pituitaries of these fish, including those of the pars intermedia, showed any apparent change after metopirone treatment.

The cytology of the lead–haematoxylin-positive cells from the acthar-treated salmon did not exhibit any apparent histological changes from the controls (Fig. 14 and 16).

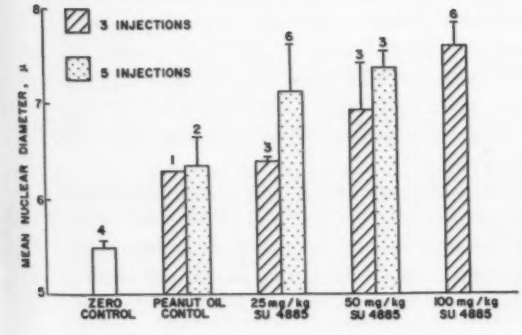

FIG. 22. Effect of metopirone on mean interrenal nuclear diameters (treatment mean + SD) in rainbow trout. Numbers of fish are indicated by figures above the bars. Correlation coefficient for values from fish receiving three injections of 25, 50, and 100 mg of SU 4885: $r = 0.85$.

DISCUSSION

Female castrated salmon receiving metopirone had lower plasma cortisol concentrations than control fish which received peanut oil injections. In addition, cortisol concentrations were much lower in metopirone- than in ACTH-treated fish, although histological examination showed that the adrenals were stimulated to similar extents in both groups. The evidence thus indicates that metopirone was inhibiting 11β-hydroxylase activity in the interrenal of these fish. The relatively high concentrations of cortisol observed in the control fish may have been a result of a stressful effect of the peanut oil, or of the daily injection procedure itself. The increased mean interrenal nuclear diameters in the metopirone- and ACTH-injected salmon, compared with their respective controls, show that the interrenal response to metopirone is probably mediated by pituitary ACTH released in the absence of cortisol feedback inhibition.

In the trout experiments, three daily injections of 25 or 50 mg/kg metopirone resulted in cortisol concentrations higher than those observed in peanut oil or zero time control groups, suggesting that the major effect of metopirone at this dosage may have been that of a stressor, rather than an 11β-hydroxylase inhibitor. However, five injections at these dosages, or three injections of 100 mg/kg, resulted in mean cortisol concentrations lower than those of the control groups, showing that 11β-hydroxylase inhibition was occurring. The trout receiving larger doses, or more injections, had the highest mean interrenal nuclear diameter. Thus, the degree of stimulation received by the pituitary corticotrophs and, therefore, ACTH production, was related to the total amount of metopirone injected.

The presence of a pituitary–interrenal axis in *Salmo gairdneri* was demonstrated by Donaldson and McBride (1967) who found a decrease in interrennal nuclear diameter and plasma cortisol concentration after hypophysectomy. The stimulation by metopirone of the interrenals of sockeye salmon and trout was accompanied by increased activity of one cell type only in the pituitaries, namely the palisade-like strand of cells located between the rostral pars distalis and the neurohypophysis. Furthermore, these were the only cells of the pars distalis that were specifically stained with lead–haematoxylin. The parallelism between the heightened activity of the adrenocortical cells after metopirone administration, and the stimulation of the lead–haematoxylin cells of the pars distalis, strongly suggests that these cells may be the corticotrops. Thus, these findings are in agreement with Olivereau who identified a similar cell type as the corticotrop in *Poecilia* (Olivereau and Ball, 1936b, 1966) and in the eel (Olivereau, 1965).

Olivereau (1965) found in experiments with eels (*Anguilla anguilla*) that some animals died during metopirone treatment and all became aesthenic. Mortalities also occurred in the present experiments, the number of deaths being to some degree dose-dependent, and occurring in salmon and trout injected with metopirone ditartrate in aqueous solution. That metopirone may

be a stressor in animals has been indicated by Ganong and Gold (1960) who found that the drug evoked an increase in blood ACTH levels in adrenalectomized dogs.

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Uptake of Sodium and Phosphorus, and Weight Changes in Prerigor Cod Muscle Dipped in Sodium Tripolyphosphate Solutions[1]

By A. H. Sutton and J. M. Ogilvie

Unilever Research Laboratory
Greyhope Road, Aberdeen, Scotland

ABSTRACT

The uptake of sodium and phosphorus and weight changes in small samples (ca. 10 g) of prerigor cod (*Gadus morhua*) muscle dipped in sodium tripolyphosphate ($Na_5P_3O_{10}$ $6H_2O$) solutions have been measured. These parameters were studied as a function of solution concentration for a constant dipping time of 2 min. The uptake of solution and quantity of drip produced on thawing decreased between 0 and 7 hr postmortem, partly compensating one another leaving the final muscle weights the same for the two periods. Calculations show that the additional sodium and phosphorus in the muscle after dipping and after freezing and thawing to be in the same ratio as that in solution, indicating that the $Na_5P_3O_{10}$ molecule remains intact during processing under the conditions used here. About 260 moles of water per mole of $Na_5P_3O_{10}$ are retained in the muscle after freezing and thawing.

INTRODUCTION

Polyphosphates are used as additives to fish muscle to maintain the natural water content of the muscle during storage, and in some instances to reduce drip loss. There is a considerable volume of literature on the commercial application of the polyphosphates in the fishing industry (e.g. Chalker et al., 1965; MacCallum et al., 1964; Boyd and Southcott, 1965; Tanikawa et al., 1963), and more recently a few papers have appeared on the distribution of the phosphates and mechanism of their reaction (Love and Abel, 1966; Kuusi et al., 1965). However, there has been little, if any, effort to precisely quantify the changes taking place during the dipping, freezing, and thawing cycle. This is a nessary basis for a detailed investigation into the process, which when understood, should lead to the formulation of more efficient treatments. Some aspects of the sodium tripolyphosphate reaction with cod muscle (*Gadus morhua*) are described in this paper.

The complete characterisation of the system is lengthy because of the large number of variables involved, and the present study has been limited to an investigation of the effect of the solution concentration on the muscle uptake for a constant dipping time of 120 sec. The experiments were carried out at two periods after death, immediately after killing and filleting, and after 7 hr on ice, as this is the period of interest with fish which are processed and frozen at sea. The weight changes after dipping (freezing) and thawing

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and the concentration changes in sodium and phosphorus were measured, and from these results the water/salt relationships in the muscle have been calculated.

Since the experiment was designed for studying the muscle/polyphosphate reaction, for convenience small samples of muscle were used, and therefore the weight changes should not be taken as typical of what might be obtained in commercial practice. The actual reaction will, of course, be the same.

MATERIALS AND METHODS

The sodium tripolyphosphate (TPP) (food grade), manufactured by Allbright and Wilson, England, has a typical analysis of 91.5% $Na_5P_3O_{10}$ $6H_2O$. The solutions were prepared by dissolving the salt in cold, distilled water, with continuous agitation. All the tests were carried out at the natural *p*H of the solutions, which varied from 8.9 to 9.4 for the concentrations 1, 2, 4, 6, and 8% w/v.

The cod were caught in the North Sea off Aberdeen, brought back alive, and kept in outdoor concrete tanks until required. The fish were killed by a blow on the head, and filleted. One fillet was processed immediately, and the other held on ice for 7 hr. By using small samples (about 10 g) only three fish were required for each period after death. Cubic-shaped sections were cut from the middle one-third portion of the fillet, and processed as follows after 0 and 7 hr on ice.

1. The muscle was weighed, dipped in the reagent, removed, and reweighed immediately (W_1, Tables I and II). This gives the weight of the surface film of solution.

2. The muscle was suspended in the reagent for 120 sec, removed, and reweighed (W_2, Tables I and II). This gives the total weight of fluid taken up.

3. The muscle was then frozen for 24 hr at −29 C, and thawed for a further 24 hr at 18 C and reweighed (W_3, Tables I and II). To collect the fluid separating after thawing the sample, a method was required which would ensure (1) no occurrence of drying of the sample; (2) a complete separation of drip from muscle; and (3) no abnormal pressure effects. We found that a technique based on the method of Kaloyereas (1947) was suitable. A length of polythene tubing was attached to a 3-inch diam funnel, and sealed off with a screw clip. A piece of wire mesh (1 inch diam) was placed inside the funnel, which was then filled with paraffin. Several such units were constructed, and were kept at a constant temperature of 18 C. In use the sample was placed on the wire mesh, the drip over 24 hr separated and collected, and the muscle lightly blotted and reweighed. It was assumed that no absorption of paraffin occurred.

4. The muscle and drip were then analysed. For the chemical analyses, samples of the muscle and fluid were ashed at 520 C and the residue dissolved in water. Recovery experiments have shown that no loss of either Na or P occurred under these conditions. The sodium was measured directly on a

flame photometer and the phosphorus estimated colormetrically as the phosphovanadomolybdate (Hanson, 1950).

For each experiment one fillet was used for dipping tests and the other processed as the undipped controls. All weight changes are expressed relative to an original starting weight of 1 g of muscle.

RESULTS

The weight changes during processing are shown in Tables I and II, and the sodium and phosphorus concentrations in the muscle and drip in Tables III and IV. The data for the three fish are given, together with the mean values in parentheses. The scatter of the results for the triplicate experiments appears to be random and not due to consistent variations in the state of the three fish. Agreement is considerably better at 7 than at 0 hr, but in all cases the mean values have been used for subsequent calculations. The weight of absorbed fluid (W_2–W_1), drip W_4 (W_2–W_3, or for controls 1–W_3), and muscle after thawing W_3, are shown in Fig. 1.

The fairly constant quantities of absorbed fluid are a little surprising, since this indicates that osmotic pressure differences are not the main driving force of the reaction. Unpublished results from our laboratory show that

TABLE I. Weight changes on dipping (freezing) and thawing cod muscle at 0 hr postmortem. All weights relative to a starting weight of 1 g.

	Surface dip (g) W_1	Total dip (g) W_2	Final muscle weight (g) W_3
Undipped control	–	–	0.866 0.860 (0.845) 0.808
% TPP 1	1.022 1.037 (1.025) 1.017	1.105 1.066 (1.077) 1.060	0.929 0.902 (0.918) 0.924
2	1.105 1.012 (1.017) 1.023	1.087 1.053 (1.061) 1.041	0.941 0.863 (0.919) 0.953
4	1.019 1.024 (1.024) 1.030	1.082 1.047 (1.077) 1.013	1.011 0.819 (0.941) 0.994
6	1.027 1.010 (1.024) 1.034	1.073 1.061 (1.073) 1.085	1.025 0.926 (0.964) 0.940
8	1.049 1.054 (1.050) 1.046	1.070 1.107 (1.103) 1.124	0.986 0.989 (0.982) 0.972

TABLE II. Weight changes on dipping (freezing) and thawing cod muscle after 7 hr on ice postmortem. All weight changes relative to a starting weight of 1 g.

	Surface dip (g) W_1	Total dip (g) W_2	Final muscle weight (g) W_3
Undipped control	–	–	0.899 0.584 (0.866) 0.846
% TPP 1	1.030 1.017 (1.023) 1.022	1.032 1.066 (1.053) 1.059	0.919 0.869 (0.902) 0.917
2	1.030 1.026 (1.028) 1.029	1.038 1.055 (1.053) 1.067	0.956 0.923 (0.927) 0.901
4	1.017 1.024 (1.022) 1.026	1.063 1.064 (1.065) 1.067	0.951 0.899 (0.942) 0.975
6	1.019 1.022 (1.022) 1.026	1.069 1.054 (1.061) 1.061	1.005 0.955 (0.979) 0.978
8	1.019 1.026 (1.021) 1.017	1.043 1.086 (1.063) 1.057	0.991 0.985 (0.974) 0.946

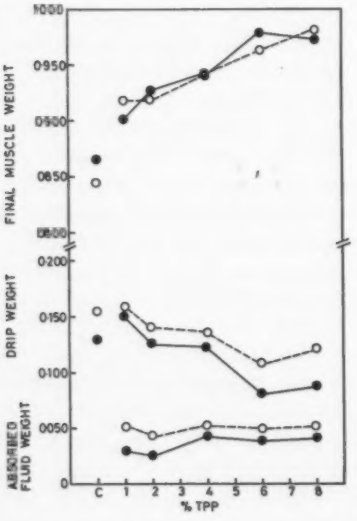

FIG. 1. Weight of absorbed fluid and drip produced on thawing, and final muscle weight vs. solution concentration. ○ = 0 hr, ● = 7 hr, C = undipped controls.

about a 6.5% TPP solution has the same osmotic concentration as the muscle (450–500 m osmolar), and on this basis alone an uptake of fluid would only occur in solutions more dilute than this. These results also show that the amount of absorbed fluid decreases with time postmortem.

The weight of drip and the final muscle weight are clearly dependent on the solution concentration. It should be noted that the control values will not be the same as obtained in a 0% TPP solution. It appears that there is no difference in yield with muscle treated 0 or 7 hr postmortem but it has been shown (Sutton, 1967) that weight losses decrease rapidly during the first 2 days storage on ice. The higher drip losses in the 0-hr group fish is also shown by the untreated controls, and is a reflection of the changing biochemical properties as the muscle progresses into rigor. The drip and weight change figures are, of course, the same for untreated controls.

Table III shows that there is a final net increase of Na in the muscle for all concentrations of dip, but that a loss in P may occur from muscle which has been dipped in the dilute solutions. The concentration of Na in the drip (Table IV) of the treated fish is always greater than the controls, but with the 7-hr postmortem dipped fish is not well related to the concentration of the dip. With the P in the drip for 0-hr processed fish, there is a slight increase with the concentration of the dip, but at 7 hr it remains fairly constant.

TABLE III. Concentrations of sodium and phosphorus in muscle after thawing (mmoles/kg) (X_2).

Hours postmortem:	Na				P			
	0		7		0		7	
Undipped control	16.2		16.5		71.3		71.3	
	16.6	(16.2)	13.2	(16.1)	69.4	(71.5)	68.1	(70.0)
	15.9		18.7		73.9		70.6	
% TPP 1	35.5		24.4		75.5		63.2	
	26.2	(27.7)	21.3	(23.2)	67.7	(73.8)	66.4	(66.6)
	21.4		23.9		78.4		70.3	
2	43.9		32.0		65.2		76.5	
	33.0	(37.5)	35.1	(33.3)	–	(66.4)	72.6	(76.5)
	35.8		32.7		67.7		80.3	
4	67.0		59.4		97.4		86.1	
	43.9	(58.7)	55.7	(62.1)	79.0	(89.3)	82.6	(89.1)
	65.2		71.1		91.6		98.7	
6	95.6		96.5		77.4		106	
	62.6	(80.3)	68.3	(84.8)	107	(94.6)	95.2	(102)
	82.7		89.5		99.4		104	
8	108		89.6		110		106	
	146	(108)	82.3	(85.7)	125	(109)	136	(116)
	71.2		85.3		92.1		106	

TABLE IV. Concentrations of sodium and phosphorus in the drip (mmoles/kg) (X_4).

Hours postmortem:	Na		P	
	0	7	0	7
Undipped control	48.7	15.6	52.9	41.0
	18.4 (26.7)	19.2 (17.0)	71.0 (55.5)	43.5 (45.6)
	13.0	16.1	42.6	52.3
% TPP 1	34.5	36.0	51.6	44.2
	30.9 (29.2)	63.5 (55.0)	41.0 (42.0)	62.9 (66.6)
	22.3	65.5	33.5	92.9
2	33.4	36.2	35.2	33.5
	51.3 (36.0)	38.1 (48.8)	87.1 (52.0)	42.2 (41.1)
	23.3	72.1	33.9	47.7
4	60.0	58.2	38.4	51.3
	69.6 (56.5)	44.0 (55.1)	69.7 (50.9)	42.0 (50.7)
	40.0	63.2	44.5	58.7
6	50.0	69.1	115.0	75.5
	83.2 (62.1)	43.4 (59.4)	53.2 (72.4)	40.6 (62.8)
	53.0	65.7	49.0	72.3
8	69.1	82.0	54.5	42.6
	99.0 (87.9)	83.5 (84.4)	91.2 (79.8)	65.2 (66.6)
	95.7	87.6	93.9	91.9

DISCUSSION

Recent results from our laboratory (unpublished) have shown that cod muscle has properties analogous to simple water-swollen gel systems, such as gelatin in water, This has also been shown with halibut muscle (Tarr, 1942), and probably for meat in general (Hamm, 1960). For example the swelling behaviour towards various salt solutions follows the Hofmeister series, and is *p*H dependent. Also, the problem we are concerned with here, the formation of runoff fluid from muscle, is similar to syneresis in gels. Thus as a first approximation we may expect changes taking place in the phosphate dipping process to be characteristic of a gel/aqueous phase reaction.

Muscle may be considered as an organised actomyosin gel system, divided up into discrete units by the cell membranes. The solid, proteinaceous structure is negatively charged, and electroneutrality is maintained by the potassium and sodium ions present in the surrounding aqueous phase. There is also a multitude of other diffusible ions and molecules distributed throughout the structure.

When muscle is immersed in the phosphate solution, a number of diffusion processes came into operation. Sodium and tripolyphosphate ions will diffuse into the muscle and the mobile species will diffuse out into the solution until the system reaches equilibrium. For muscle cubes weighing about 10 g this

takes 20–30 hr. Therefore, in processes where a whole fillet is dipped for only a few minutes, the reaction will be restricted to the surface layers, as found by Love and Abel (1966).

The total concentration of Na and P in the muscle after dipping (X_2) can be obtained from the final muscle and drip concentrations by use of the equation

$$W_4X_4 + W_3X_3 = W_2X_2 \quad \ldots\ldots(1)$$

where X_3 and X_4 are the observed concentrations in the muscle and drip respectively.

X_2 as a function of the solution concentration is shown in Fig. 2. Clearly, an uptake of Na occurs at all concentrations, whereas with P a net increase is obtained only in greater than 2% solutions. From the ratio of the slopes of the linear portions of these curves the absorbed Na:P ratio is 1.64:1 (TPP 1.67:1), indicating the absorption of the TPP molecule from dip concentrations of 2% and above.

The increase in Na and P in the muscle after dipping, ΔX_2, is given by

$$X_{2\ \text{dipped}} - X_{2\ \text{control}} = \Delta X_2 \quad \ldots\ldots(2).$$

This data is shown in Table V together with a calculated value from the total weight increase on dipping, assuming the fluid taken up to be the same concentration as the solution.

The uptake of Na is much higher than calculated from the weight of absorbed fluid, whereas the P concentrations are less for all dips in the 0-hr treatment, with a net loss of P from the muscle in the 1 and 2% solutions. With the 7-hr fish there is an increased uptake of P, and ΔX_2 exceeds the calculated values above 4% TPP. Assuming that absorption from solution

TABLE V. Changes in the sodium and phosphorus concentrations in the muscle after dipping (mmoles/kg). ($X_{2\ \text{dipped}} - X_{2\ \text{control}}$) = ΔX_2.

Hours postmortem			% TPP 1	2	4	6	8
0	Na	ΔX_2	10.1	19.5	40.6	60.5	87.0
		Calc.	8.1	12.8	32.4	46.0	86.5
	P	ΔX_2	−0.3	−4.6	15.1	23.2	36.5
		Calc.	4.9	7.7	19.4	27.6	51.9
7	Na	ΔX_2	11.8	19.0	45.0	66.5	69.4
		Calc.	5.6	11.1	27.3	38.4	53.0
	P	ΔX_2	−0.1	5.3	17.7	32.1	45.0
		Calc.	3.3	6.7	16.4	23.2	31.8

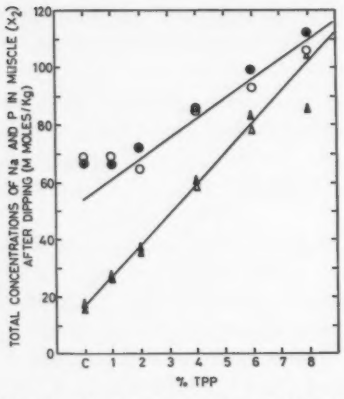

FIG. 2. Total concentrations of Na and P in muscle (X_3) after dipping vs. solution concentration. Sodium: △ = 0 hr, ▲ = 7 hr, postmortem; phosphorus: ○ = 0 hr, ● = 7 hr postmortem; C = undipped controls.

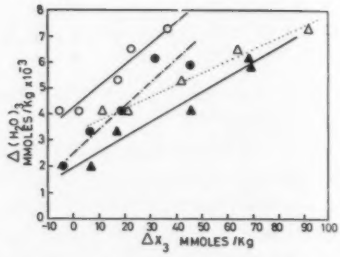

FIG. 3. Moles of water retained as a function of the retained sodium and phosphorus. Sodium: △ = 0 hr, ▲ = 7 hr postmortem; phosphorus: ○ = 0 hr, ● = 7 hr postmortem.

is occurring as TPP, then the loss of muscle P compounds must exceed the uptake of tripolyphosphate P in the dilute solutions. Extrapolation of the phosphorus curves in Fig. 2 to zero solution concentrations indicates that about 12 mmoles/kg of muscle P is lost during a 120-sec dipping process.

From the high values of absorbed Na it appears that the rate of uptake of TPP is faster than that of water, i.e. the solution will be depleted of TPP under conditions of continuous use. However, large scale experiments in our laboratory have shown that this is not a serious problem in the commercial application of the process.

The data in Fig. 1 show that the weight losses of the muscle and quantity of drip produced on thawing are reduced by the polyphosphate treatment. The fluid retention is related to the concentration of the dip, and thus to the uptake of Na and P in the muscle. This can be obtained from

$$X_{3\,\text{dipped}} - X_{3\,\text{control}} = \Delta X_3 \text{ mmoles/kg} \qquad \ldots\ldots(3).$$

The amount of fluid retained in the muscle due to the dipping process is given by

$$W_{3\,\text{dipped}} - W_{3\,\text{control}} = \Delta W_3 \qquad \ldots\ldots(4).$$

Assuming that ΔW_3 is mainly water, then this can be written as

$$\frac{1000\,\Delta W_3}{18} = \Delta(H_2O)_3 \text{ moles/kg.}$$

$\Delta(H_2O)_3$ as a function of ΔX_3 has been plotted in Fig. 3.

Within the limits of experimental error these appear to be straight-line relationships where the slope r is the moles of water retained per mole of Na or P. If $r_{Na} = \Delta(H_2O)/\Delta(Na)$ and $r_P = \Delta(H_2O)/\Delta(P)$, then $r_P/r_{Na} = \Delta(Na)/\Delta(P)$. r_{Na}, r_P, and r_P/r_{Na} are given below

Hours postmortem	r_{Na}	r_P	r_P/r_{Na}
0	47	81	1.7
7	57	91	1.6

Na:P ratio in TPP = 1.67

The values for r_P/r_{Na} indicate that the retained ions in the muscle are present as TPP. The mean moles of water retained per mole of TPP is therefore given by $(5r_{Na} + 3r_P)/2$, and this is 240 moles for 0-hr and 280 for 7-hr treated. fish. Since this difference is within the experimental error, a value of 260 moles of water per mole of TPP can be taken as a reasonable figure.

CONCLUSION

The results reported here show that the uptake of ions into cod muscle during dipping occurs in the same ratio as they are present in solution, i.e. as TPP, $Na_5P_3O_{10}$. They are also retained in the muscle after freezing and thawing in the same ratio, and the increase in fluid retention is proportional to their concentration. It appears that an exchange reaction occurs during dipping between the polyphosphate anion and the muscle phosphate, giving a net loss of P from the muscle in the dilute solutions.

Calculations show that for each mole of TPP in the muscle after thawing there are about an additional 240 moles of water compared with the undipped controls.

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Adjustment of Buoyancy by Longnose Dace (*Rhinichthys cataractae*) in Relation to Velocity of Water[1]

By J. H. Gee

Department of Zoology
University of Manitoba, Winnipeg, Man.

ABSTRACT

The buoyancy of longnose dace (*Rhinichthys cataractae*) is correlated with the velocity of water in which they are living. Swimbladders from fish collected in rapids of streams and rivers are smaller in volume than in those fish collected from still water. Laboratory experiments on yearling and older fish show that individuals alter their buoyancy by changing the volume of their swimbladder. Buoyancy depends on velocity of water and size of fish. Longnose dace adjust their buoyancy rapidly in response to changes in velocity of water. They can decrease their buoyancy to adapt to an increase in velocity of water in 3 hr. A complete adjustment requires 4 days. Fish with access to the surface can increase their buoyancy in response to reduced velocities by completely inflating their swimbladders in less than 24 hr. Fish without access to the surface require 48 hr to complete the same adjustment. Thus, longnose dace have a functional gas gland even though they are physostomes. The gas gland does not function during the early stages of life after hatching. Alevins come to the surface to fill initially their swimbladders. The ability to adjust buoyancy rapidly is an adaption to life in rivers and streams by fishes that live mostly in swift-flowing waters but are sometimes forced to live temporarily in reduced current when the volume of water flowing is diminished.

INTRODUCTION

The role of the swimbladder as a hydrostatic organ in fishes was examined with reference to vertical movements by Jones (1951, 1952, 1957). The ability of stream-dwelling fishes to adjust their buoyancy by altering the volume of their swimbladder to cope with changing velocities of water has received attention only recently (Saunders, 1965; Neave et al., 1966). This study seeks to determine the effect of different velocities of water on the buoyancy of the longnose dace, *Rhinichthys cataractae* (Valenciennes).

The longnose dace is a cyprinid commonly found in lakes and streams across North America. It occurs as far south as the Rocky Mountains in Mexico and as far north as the Mackenzie River near the Arctic Circle (Carl et al., 1959). Its ecology in the Fraser River, British Columbia, was studied by Gee and Northcote (1963). Newly emerged fry live in still, shallow waters along the shore and adult fish occur mainly on the bottom in spaces between stones in fast-flowing waters. Aquatic insect larvae and nymphs are the main item of diet of fish of all ages.

Bailey and Allum (1962) found that the swimbladder of adult longnose dace was variable in size. The length of the swimbladder expressed as a percentage of the standard length of the fish varied from a mean in one sample

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of 19.1% to 32.3% in another sample. These authors suggested that fish with large swimbladders may live in pools of rivers and streams or in lakes, where near-neutral buoyancy would be advantageous, whereas those with smaller swimbladders are found in fast velocities of water where a negative buoyancy would aid in holding a position on the bottom.

The object of this paper is to determine (1) if a correlation exists between buoyancy and velocity of water in longnose dace collected from the wild; and (2) whether they adjust their buoyancy in response to varying velocities of water in the laboratory and if so, to learn something of the rates at which and methods by which such changes occur.

MATERIALS AND METHODS

Collections of dace from Fraser river below Quesnel, B.C., the Cottonwood River, B.C., Moose Lake in northern Manitoba, and the Roseau and Rat rivers in southern Manitoba were examined (1) to determine the variability in volume of the swimbladder; and (2) to correlate this variability with the velocity of water in which the fish lived. Fish were preserved in 10% formalin.

The volume of the swimbladder was measured by inflating the collapsed bladder in a water-filled glass tube (Fig. 1) and recording the volume of displaced water. The swimbladder was removed from the fish and the point of a needle of a syringe inserted into the posterior lobe of the swimbladder. The swim-

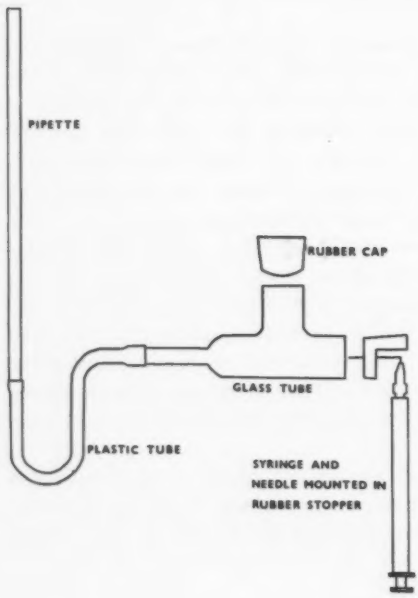

FIG. 1. Apparatus for determining the volume of the swimbladder of preserved fish.

bladder was completely deflated before being placed into a glass tube where it was held in position by a rubber stopper through which the needle of the syringe passed (Fig. 1). Water was added through the top of the glass tube until both the plastic tube and the lower end of the pipette were filled. A rubber cap was placed in the top opening of the glass tube. Care was taken to ensure that no bubbles of air were present in either the glass tube, the plastic tube, or the pipette. Then the swimbladder was completely inflated by the syringe. The volume of the swimbladder was obtained by measuring the volume of water displaced in the pipette.

Experiments were completed in the laboratory to determine if longnose dace adjust the volume of their swimbladders and the rate at which such adjustments are made. Fish were collected for these experiments by seining several sections of rapids of the Roseau River in October and November 1966. Fish were held in still water in large plastic containers (40 × 59 × 49 cm) for 1–2 months before the experiments. Prior to and during the experiments, fish were held in a water temperature of 15 C (±1), with a normal photoperiod for the particular season. Food consisted of frozen brine shrimp and small enchytraeid worms.

A factorial experiment was designed to determine whether buoyancy of longnose dace depended on size of fish, velocity of water, or both. Fish were divided into three groups based on standard length: 20–31, 32–44, and 56–78 mm. Each size-group of 24 fish was divided into two batches of 12 fish each. One batch was kept in a current of water of about 30 cm/sec, the other in still water. Each batch was held in a rectangular aquarium, 170 × 140 mm, filled to a depth of 240 mm.

The current of water was created by placing two air stones at opposite corners on the bottom at one end of an aquarium. Bubbles from these air stones created a circular current on a vertical plane. A layer of stones 2–8 mm in diameter was placed on the bottom to provide areas of reduced flow. Fish in these aquaria remained mostly on the bottom and swam against the current.

Buoyancy of fish in each batch was determined after 10 days. Buoyancy was measured in terms of floatation pressure (Saunders, 1953). Floatation pressure is obtained by dividing the weight of the gas-free fish in water (in grams) by the volume of gas in the swimbladder (in milliliters).

On the basis of this first experiment, two additional experiments were made to determine the rate at which dace adjust their buoyancy by altering the volume of their swimbladder. To reduce variation in volume of swimbladder found between fish of different sizes, only those fish between 20 and 50 mm long were used in these experiments. In the second experiment, the ability of dace to decrease the volume of their swimbladders was measured. Fifty-six fish were held in still water for 14 days. They were then divided into seven groups with eight fish per group. The floatation pressure of fish in one group was measured after 14 days in still water. The remaining groups, after 14 days in still water, were transferred to a water velocity of about 30 cm/sec.

Each group was kept in water current for a different interval of time (3, 6, 12, 24, 48, and 96 hr), and then floatation pressures determined.

The third experiment compared the ability of fish to inflate their swimbladders with and without access to the surface after fish had been held in current and then transferred to still water. One hundred and twelve fish were held in current of about 30 cm/sec for 14 days. These fish were divided into seven groups of 16 fish each. Each group was further divided into two batches of eight fish each and each batch placed into an aquarium. One batch of each group had access to the surface, the other did not. The floatation pressure of fish in one group was measured after 14 days in water current. The floatation pressures of fish in the remaining groups, which had also been in water current for 14 days, were measured after being placed in aquaria with still water for either 3, 6, 12, 24, 48, or 96 hr.

Fish without access to the surface were held in aquaria covered on the top by a nylon screen of 3-mm mesh diam and completely emersed below the surface of the water in a large container. Fish with access to the surface were held in aquaria placed in a similar container but one that did not contain water. The resulting depth of water for fish without access to the surface was 260 mm and for those with access to the surface the depth was 240 mm.

The method by which newly hatched dace initially filled their swimbladders was observed. Newly hatched alevins from eggs spawned in the laboratory were placed in two plastic trays. The bottom and top of each tray was cut out and replaced with a fine-meshed screen (16 meshes/cm). One tray was suspended at the surface of the water in a large container, the second just below the surface. When fry were 9–16 days old, they were anesthetized with tricane methanesulfonate and examined to determine if the swimbladder, visible through the transparent tissues, was inflated.

RESULTS

Volume of Swimbladder Related to Velocity of Water

The volume of swimbladders of fish were measured in collections of longnose dace taken from different environments to determine if the volume of the swimbladder was related to the velocity of water in which the fish was living.

Figure 2 shows the relationship between volume of swimbladder and weight of individual fish from the Fraser River (Gee and Northcote, 1963). Two features are apparent. First, after the fish reach a weight of about 1.0 g, the volume of the swimbladder increases little with age and increasing weight. The majority of swimbladders from fish weighing between 1.0 g to the largest of almost 30.0 g differed little in volume, between 0.01 and 0.08 ml. Most likely, density of these fish in water becomes greater and buoyancy decreases as they increase in weight. Secondly, there were two exceptions. Two fish weighing 10.5 and 13.7 g had swimbladders whose volume greatly exceeded those of other fish of a similar size. These volumes were 0.195 and 0.190 respectively. The swim-

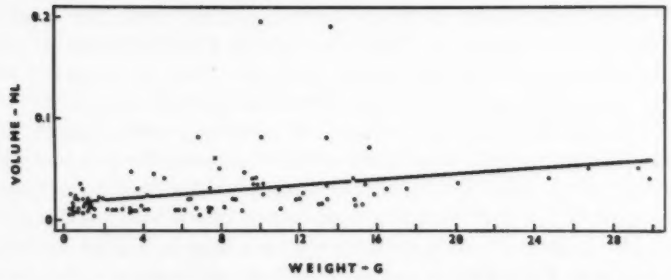

FIG. 2. Relationship between volume of swimbladder and weight of fish collected from the Fraser River. From Gee and Northcote (1963). The regression is significant at $p < 0.01$; correlation coefficient 0.30.

bladder of longnose dace varies, therefore, not only with size but also between individuals of any one age or size.

Variability in volume of swimbladder between individuals of a similar weight is shown strikingly in Fig. 3, which is based on fish from the Cottonwood River in July 1956. Two almost distinct groups of fish exist: those with small swimbladders similar to the majority of fish in the collection from the Fraser River, and those whose swimbladders are much larger in volume.

These fish were collected from both rapids and pools, but were not kept separate. Possibly those individuals with small swimbladders and a negative buoyancy were taken from the rapids, whereas those with large swimbladders and a near-neutral buoyancy were taken from still water.

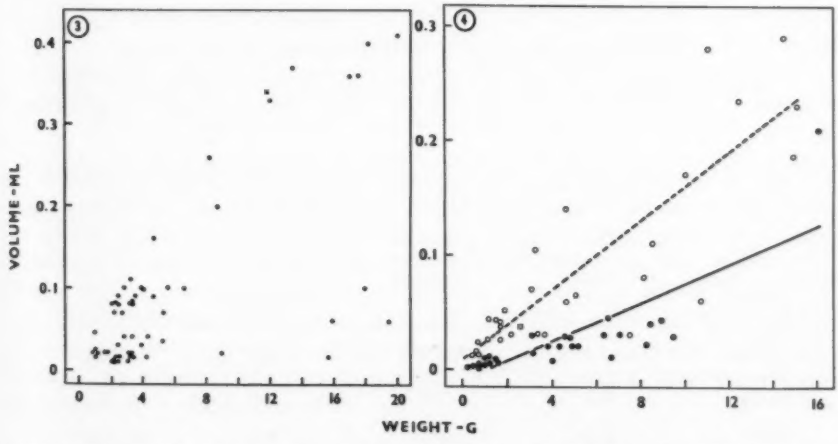

FIG. 3. Relationship between volume of swimbladder and weight of fish collected from the Cottonwood River, B.C.

FIG. 4. Relationship between volume of swimbladder and weight of fish from Moose Lake (open circles) and from the Roseau and Rat rivers (solid circles).

To test this possibility, volumes of swimbladders were measured on fish collected during the summer of 1963 from Moose Lake (still water) and from several sections of rapids of the Roseau and Rat rivers in August 1966. The majority of longnose dace from Moose Lake had swimbladders whose volumes were much larger than the majority of fish collected from Roseau and Rat rivers (Fig. 4). Regression lines, calculated for fish from the two environments are significant ($p < .01$). The correlation coefficient for fish from Moose Lake was 0.86 and 0.81 for fish from the Roseau and Rat rivers. A test of homogeniety on the regression coefficients of the two lines indicated heterogeniety ($p < .005$). Thus the slopes of the two lines are different.

Adjustment of Buoyancy

Buoyancy dependent upon size of fish and velocity of water

The analysis of variance on the results of the factorial experiment with two water velocities, still and running, and three size-groups of fish showed that the main effects of size and velocity of water were significant, as was the interaction between these two factors ($p < .005$). Differences in floatation pressure between fish held in still and running water depended on the size of fish.

Table I shows that fish of all size-groups held in still water had a significantly higher floatation pressure than fish held in water current but the difference between water velocities for the 56–78-mm size-group was less than that of the two smaller size-groups.

Of the fish held in still water, those of the 20–31- and 31–44-mm size-group had a significantly higher floatation pressure than did fish of the 56–78-mm size-group. The difference between the two smaller size-groups held in still water was not significant (Table I). A similar relationship was found between the three size-groups held in water current.

This experiment shows that longnose dace can adjust their buoyancy by changing the volume of their swimbladder. The volume of the swimbladder

Table I. Effect of size of fish and velocity of water on the floatation pressure of longnose dace. Probabilities are from the least significant difference based on error variance.

Size of fish (standard length, *mm*)	Mean floatation pressure[a]		Difference	*P*
	Still water	Running water		
20–31	.972 *[b]	.328 *	.644	<.001
31–44	.869 *	.299 *	.570	<.001
56–78	.511	.141	.370	<.001

[a]Mean based on 12 fish.
[b]Significantly different from those indicated by the arrow ($p < .01$); from Duncan's New Multiple Range Test.

and the resulting buoyancy is dependent upon size of fish and velocity of water in which the fish are living.

ABILITY TO DECREASE BUOYANCY

Fish which had been held in still water and then transferred to running water made a rapid decrease in their buoyancy. An initial adjustment was made during the first 3 hr of exposure to running water (Fig. 5). After this period the rate of change was much slower. After 96 hr in running water, fish had reached a minimum floatation pressure similar to that of fish of a comparable size-range that had been in water current for 10 days. Appendix I gives the range of variation and 95% confidence limits of the means illustrated in Fig. 5.

ABILITY TO INCREASE BUOYANCY

Fish could increase their buoyancy rapidly when transferred from running to still water with and without access to the surface. But those with access to the surface could do so faster than those without (Fig. 6). Fish with access

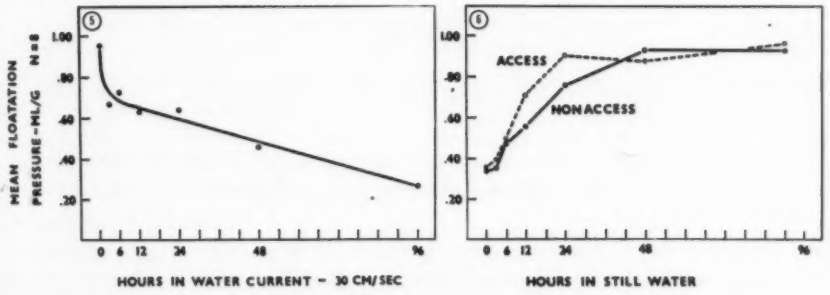

FIG. 5. Relationship between floatation pressure and time in running water.

FIG. 6. Relationship between floatation pressure and time in still water.

to the surface reached near-neutral buoyancy after 24 hr in still water whereas those without access required 48 hr. Appendix II gives the range and 95% confidence limits on the means illustrated in Fig. 6. Regression lines were calculated for the rates of increase in floatation pressure for fish with access to the surface during the first 24 hr and for fish without access during the first 48 hr. The regression lines are $y = .354 + .0239x$ and $y = .373 + .0124x$ respectively. Both regressions are significant ($p < .01$). A significant difference between the slopes of the two lines was shown by a test of homogeniety on the regression coefficients ($p < .01$).

INITIAL FILLING OF THE SWIMBLADDER BY ALEVINS

The object of this experiment is to determine when and how the swimbladder is filled initially by newly hatched alevins.

Newly hatched alevins were placed in two small trays, one at the surface and one below the surface in large containers of still water. These fish were anesthetized at 9–16 days of age. Of 56 fish which had access to the surface, 51 had swimbladders filled with gas. The average length of the inflated bladders was 10.9% of the standard length of the fish. None of the 15 fish without access to the surface had gas in its swimbladder. Only the posterior lobe of the swimbladder was inflated at 9–16 days of age in those fish with access to the surface. The anterior lobe was not observed to be inflated until fish were at least 3-weeks old. However, fish with only the posterior lobe inflated were neutrally buoyant and occupied a position near the surface of the water.

After the alevins without access to the surface were examined and had recovered from the anesthetic, they were observed darting to the surface where they filled their swimbladders. These fish were again examined 20–50 min after recovery and 8 of the 15 were found to have gas in their swimbladders. In addition, seven fish were observed to have bubbles of gas in their alimentary canal suggesting that alevins of longnose dace initially fill their swimbladders by gulping air at the surface of the water and passing it along the pneumatic duct to the swimbladder.

DISCUSSION

Relationship between Buoyancy of Stream-Dwelling Fishes and Velocity of Water

The advantages of neutral buoyancy in still water and negative buoyancy in fast velocities of water were discussed by Gee and Northcote (1963) and by Saunders (1965).

Variation in buoyancy occurs between closely related species and is correlated with velocity of water. Gee and Northcote (1963) found in the Fraser River that whereas yearling and older longnose dace occupied swift-flowing rapids, leopard dace (*Rhinichthys falcatus*) of the same age-groups were predominately in noncurrent areas of the river. The volume of the swimbladder of adult leopard dace was much larger than that of adult longnose dace, but swimbladder volumes of newly emerged fry of both species were similar. Fry of both species occur together in shallow still waters along the shores of the Fraser River and are neutrally buoyant.

Saunders (1965) found that brook trout occupying deep pools were more buoyant than Atlantic salmon parr which are found predominately in swift-flowing rapids. Similarly, fishes of the genus *Garra* which live in swift-flowing streams have smaller swimbladders than those species from streams with lower water velocities (Hora, 1922). Nelson (1961) reported that the swimbladder of catostomids is usually 35–45% of the standard length of the fish, but individuals of the genera *Hypentelium*, *Thoburnia*, and *Pantosteus* which live in rapids have smaller swimbladders than the genera living more typically in still waters.

Adjustment of Buoyancy by Rapids-Dwelling Fishes an Advantage

These experiments show that longnose dace adjust their buoyancy. Buoyancy of yearling and older fish depends on size of fish and velocity of water. Atlantic salmon parr can also adjust their buoyancy (Saunders, 1965). Because these fishes change the volume of their swimbladder they are capable of attaining either negative or near-neutral buoyancy. Such flexibility means that they can successfully occupy a wide range in velocities, from fast-flowing rapids to pools. This ability to adjust buoyancy provides these fishes with an advantage considering that fishes occupying fast-flowing rapids of streams are subjected to many changes in their environment. Some of the most severe changes are associated with variation in volume of water flowing in the river or stream. A reduction in flow of water can result in a reduced area of rapids. Under such conditions, fishes living in such areas may be forced to move to pools, the areas of which may be reduced by a much lesser extent. Movements by Atlantic salmon parr from rapids to pools associated with reduced flow of water were found by Saunders and Gee (1964).

But the successful occupancy of pools by rapids-dwelling fishes may be dependent upon the composition of species of fishes in the pools. In the Fraser River where leopard dace and longnose dace occur sympatrically, yearling and adult longnose dace were rarely collected from noncurrent areas where leopard dace predominate. However, the occurrence of longnose dace with large and small swimbladders in the Cottonwood River suggests that these fish were taken from pools and rapids. Leopard dace were not collected in the Cottonwood River. Thus longnose dace may, in the absence of leopard dace which is a morphologically. similar species, extend its distribution to occupy still waters.

When Atlantic salmon parr and brook trout occur together in the same stream, Atlantic salmon predominate in fast-flowing water and brook trout in pools. When brook trout are either rare or absent in the stream, Atlantic salmon parr occur in both pools and rapids (Saunders, 1965).

Adjustment of buoyancy by a particular species may be a response to a change in environment which occurs at a particular time in the life cycle of the fish. Newly emerged longnose dace in the Fraser River occupy shallow still water along the shore. Later, in their 1st year, these fish gradually select faster and faster velocities of water. A change from neutral to negative buoyancy accompanies this change in environment. Atlantic salmon parr occupy riffle areas and are negatively buoyant whereas smolts on their downstream movement show an increased buoyancy (Saunders, 1965). Such a change in buoyancy facilitates downstream migration (Saunders, 1965) and is a necessary preparation for pelagic life in the sea.

Rate of Adjustment of Buoyancy

Longnose dace from the Roseau River adjust the volume of their swimbladders to attain a striking range of buoyancy. The buoyancy of these fish

depends on velocity of water and size of fish. Neave et al. (1966) found that buoyancy of Atlantic salmon parr depended not only upon velocity of water but also on the time of year.

Adjustments in buoyancy by longnose dace are completed in 1–4 days, and partial adjustments are made in 3 hr or less. Saunders (1965) found that Atlantic salmon parr and brook trout could adjust their buoyancy in 14 hr when transferred from fast- to slow-moving water and vice versa. A complete adjustment took 36 hr.

Although longnose dace are physostomes, they inflate their swimbladders rapidly even when denied access to the surface. Presumably these fish possess a functional gas gland to make such an adjustment in buoyancy. Thus, longnose dace if displaced from a swift-flowing area of a stream into a pool could attain neutral buoyancy without having to swim to the surface.

However, the gas gland does not function during the early stage of life of the longnose dace. Initial filling of the posterior lobe of the swimbladder which occurs about 9 days after hatching is by gulping air at the surface of the water. It is unknown if longnose dace alevins can inflate their swimbladders if denied access to the surface of the water for longer than 6–9 days. Tait (1960) found that the initial filling of the swimbladder of *Salmo trutta*, *S. gairdneri*, *Cristivomer namaycush*, and *Coregonus clupeaformis* was associated with the disappearance of the yolk sac and the beginning of the free-swimming and food-seeking stage of life. None of the above species can fill their swimbladders initially unless they have access to the surface of the water, even after 56 weeks without access after hatching.

A rapid decrease in buoyancy, associated with movements from still water into areas of water current, is also advantageous to longnose dace. Such movements might be made either to occupy recently inundated rapids areas brought about by an increase in volume of flow of water or to spawn as spawning by longnose dace occurs in swift-flowing rapids.

Although a reduced swimbladder volume and negative buoyancy is a well-known advantage for fishes living in swift currents, the ability to adjust the volume of the swimbladder so as to attain a range of densities in water is equally advantageous. A variable buoyancy enables fish to occupy potentially a wide range of water velocities in streams where suitable areas to live are constantly changing with the volume of flow. To be adapted completely to life in streams and rivers, fish which predominately occupy rapids must be prepared for the changes that occur in such areas. The ability to adjust buoyancy is one adaptation possessed by longnose dace to life in streams and rivers.

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(APPENDIXES I and II *follow*)

APPENDIX I. Mean and range of floatation pressures measured on seven groups of eight fish. Each group was held for a different period in water current after being transferred from still water.

Hours in water current	Floatation pressure		Standard deviation	95% confidence limits on mean
	Mean	Range		
0	.946	.888–1.000	.049	.905–.987
3	.674	.300–.767	.154	.545–.803
6	.718	.500–.962	.155	.588–.848
12	.634	.516–.786	.094	.555–.713
24	.642	.472–.822	.118	.543–.741
48	.467	.273–.630	.156	.337–.597
96	.269	.133–.467	.102	.184–.354

APPENDIX II. Mean and range of floatation pressures measured on seven groups of 16 fish in each. Eight fish in each group were allowed access to the surface of the water, the remaining eight were not.

Hours in water current	Accessibility to surface	Floatation pressure		Standard deviation	95% confidence limits on mean
		Mean	Range		
0	Access	.354	.214–.571	.126	.249–.459
	Nonaccess	.339	.108–.630	.174	.193–.585
3	Access	.392	.140–.545	.123	.289–.495
	Nonaccess	.355	.269–.542	.090	.270–.420
6	Access	.494	.197–.750	.290	.251–.737
	Nonaccess	.470	.250–.706	.173	.325–.615
12	Access	.707	.333–1.000	.238	.508–.906
	Nonaccess	.554	.429–.714	.133	.443–.665
24	Access	.900	.600–1.000	.140	.783–1.017
	Nonaccess	.753	.523–1.000	.176	.606–.900
48	Access	.873	.660–1.000	.121	.772–.974
	Nonaccess	.920	.630–1.000	.171	.777–1.063
96	Access	.968	.889–1.083	.065	.914–1.022
	Nonaccess	.924	.532–1.000	.162	.788–1.160

NOTES

Effect of Hypophysectomy on Plasma Osmolarity in Goldfish and its Reversal by Ovine Prolactin and a Preparation of Salmon Pituitary "Prolactin"

Burden (1956) showed that hypophysectomized *Fundulus heteroclitus* die of hypocloremia if kept in fresh water. *Fundulus* pituitary brei was completely effective for maintaining life in these fish. Perch pituitary was partially effective and pollack pituitary was not effective. Pickford and Phillips (1959) found that highly purified mammalian prolactin maintained life in hypophysectomized *Fundulus*. Working on the assumption that the fish pituitary contains a prolactin-like hormone, Pickford et al. (1965) attempted to extract a prolactin-like hormone from carp pituitaries using a variation of the alkaline ethanol method of Bates and Riddle (1935). Injections of this fish prolactin preparation or injections of lyophilized carp pituitary failed to prevent hypophysectomized *F. heteroclitus* from dying in fresh water.

The endocrine control of fresh water tolerance in the Teleostei has been reviewed by Schreibman and Kallman (1966). These authors concluded that the only fish that have definitely been shown to survive in fresh water after hypophysectomy are the goldfish, *Carassius auratus* (Chavin, 1956; Yamazaki, 1961), and the European eel, *Anguilla anguilla* (Fontaine et al., 1949). Since then D. Butler (personal communication) has shown that hypophysectomized *Anguilla rostrata* will survive in fresh water for at least 3 months and, in this laboratory, two hypophysectomized rainbow trout (*Salmo gairdneri*) survived for 3 months in fresh water, after which they were killed for histological examination (Donaldson and McBride, 1967). Schreibman and Kallman (1966) have suggested that different osmoregulatory mechanisms may thus be operative in diverse groups of fish.

The purpose of this study was to investigate the effect of hypophysectomy on osmoregulation in the goldfish and to determine whether a fish prolactin could be extracted from chinook salmon (*Oncorhynchus tshawytscha*) pituitary glands which had biological activity in the goldfish.

Materials and methods.— Goldfish were obtained from Hartz Mountain Pet Supplies Ltd., Vancouver, B.C. The fish ranged in weight from 18 to 38 g and were of mixed sex. They were fed commercial trout pellets of appropriate size (Clarke Co., Salt Lake City, Utah). Hypophysectomy was accomplished by the opercular approach (Yamazaki, 1961). The hypophysectomized fish were kept in 0.25% NaCl solution at 15 C. All the hypophysectomized fish were checked for completeness of the operation at the end of the experiments.

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Chinook salmon pituitaries were collected at the Spring Creek and Little White Salmon national fish hatcheries, Washington. The pituitary glands were frozen over solid carbon dioxide during transportation to the laboratory and then kept at −30 C. An acid acetone powder (AAP) was prepared from the pituitaries according to the method of Li (1952). Fifty grams of frozen pituitary glands were homogenized in an Omni-mixer (Sorval Inc., Norwalk, Conn.) in 230 ml of acid–acetone solution (87% acetone, 11% water, 2% concentrated hydrochloric acid v/v). The mixture was stirred for 1 hr at 2 C and then centrifuged. The pellet was re-extracted by stirring in 100 ml 80% acetone for 30 min followed by centrifugation. The combined supernatant solutions were added to 1500 ml cold acetone. The resultant precipitate was washed twice with acetone and dried under vacuum. The injection solution consisted of 16 mg AAP per ml of 0.6% saline.

The mammalian prolactin preparation used was Prolactin, Ovine, NIH-P-S8 (28 IU-mg) at a concentration of 7.15 mg per ml of 0.6% saline.

In experiment I, blood was collected by inserting a 26 G ½-inch hypodermic needle into the dorsal aorta between the anal and caudal fins. A small amount of blood was drawn into the hub of the needle, using a syringe. The blood was collected from the hub of the needle, using a heparinized microhaematocrit tube. The blood was centrifuged at 2 C and 3000 *g* for 10 min. The osmolarity of the plasma was calculated from its freezing point determined by the method of Ramsay and Brown (1955).

In experiment II, blood samples were collected in heparinized tubes from the cut end of the dorsal aorta after the tail had been severed. The blood was centrifuged as described above. The osmolarity of the plasma was determined in a vapour pressure osmometer (Hewlett Packard (Canada) Ltd.) fitted with Hamilton syringes. The instrument was calibrated with standard sodium chloride solutions.

In experiment I, 12 hypophysectomized goldfish were injected intraperitoneally with 1 IU ovine prolactin per gram BW per day for 4 days, while being held in 0.25% sodium chloride solutions. A control group of 12 hypophysectomized goldfish received 0.005 ml 0.6% sodium chloride per gram BW per day. After the fourth injection, the fish were transferred to an aquarium containing fresh water and blood samples were collected 24 hr later.

In experiment II, 25 hypophysectomized goldfish were divided into two groups of 8 and one group of 9 fish. The experimental procedure was that used in experiment I. Two groups of fish received injections of saline or ovine prolactin. The third group, which contained nine fish, received four daily injections of 0.08 mg salmon AAP per gram BW. To determine the plasma osmolarity of intact goldfish in fresh water, blood samples were collected from a group of nine unoperated goldfish.

Results.— In experiment I the saline-injected fish had a mean plasma osmolarity of 246 ± 19 m osm/L compared with a mean plasma osmolarity of 282 ± 19 m osm/L in the fish which received ovine prolactin. The difference was significant ($p \leq 0.001$).

In experiment II the intact goldfish maintained in fresh water had a mean plasma osmolarity of 282 ± 8 m osm/L. The hypophysectomized fish injected with saline had a mean plasma osmolarity of 248 ± 8 m osm/L. The mean plasma osmolarities of the ovine prolactin- (270 ± 16 m osm/L) and salmon AAP- (288 ± 16 m osm/L) injected groups were both significantly higher than that of the saline-injected controls ($p \leq 0.001$).

Discussion.— The results of experiment I show that hypophysectomized goldfish receiving injections of ovine prolactin prior to transfer to fresh water had a significantly higher plasma osmolarity than goldfish which received injections of saline. Thus, despite the fact that hypophysectomized goldfish survive for long periods in fresh water, they show a response after hypophysectomy and injection of prolactin similar to that observed in fish which die after hypophysectomy if maintained in fresh water (see Bern, 1967, for a complete review).

Experiment II shows that, although the plasma osmotic pressure in the ovine prolactin-treated fish was significantly higher than in the saline-injected fish, the osmotic pressure in the prolactin group was still significantly lower than in the AAP group ($p \leq 0.05$). Thus, at the dosages used, salmon AAP was more effective than sheep prolactin under the conditions of this experiment.

Sheep AAP is known to contain adrenocorticotropic hormone (ACTH) in addition to prolactin (Li, 1952). ACTH is also present in AAP prepared from *Oncorhynchus tschawytscha* pituitaries (Donaldson, unpublished results). Several workers have shown that ACTH is not effective for the maintenance of hypophysectomized fish in fresh water (Burden, 1956; Schreibman and Kallman, 1966; Ball and Ensor, 1967). In view of these negative findings with ACTH, it is probable that the response of the goldfish to salmon AAP was brought about by a prolactin-like component.

We wish to thank the following for their help: Mrs Helen Dye for technical assistance; the Endocrinology Study Section of the National Institutes of Health for a supply of ovine prolactin; and the staff of the Spring Creek and Little White Salmon national fish hatcheries for providing facilities for the collection of salmon pituitary glands.

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

EDWARD M. DONALDSON
FUMIO YAMAZAKI[1]
W. CRAIG CLARKE[2]

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[1]Postdoctoral fellow, Fisheries Research Board of Canada. Permanent address: Faculty of Fisheries, Hokkaido University, Hakodate, Japan.
[2]Summer student, Fisheries Research Board of Canada.

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Présence du *Salvelinus namaycush* (Pisces: Salmonidae) dans les eaux marines de l'Arctique occidental canadien

De nouvelles données sur la présence du touladi ou truite grise (*Salvelinus namaycush*) en milieu marin ou du moins saumâtre ont été obtenues dans la région des lacs des Eskimos et dans la région sise au sud-est du golfe du Couronnement (Coronation Gulf), toutes deux situées dans l'Arctique occidental canadien. Ces données ont été récoltées en 1961, 1962, et 1965 par des équipes travaillant à bord du navire de recherches du Gouvernement du Canada, le M.V. *Salvelinus* sous la direction de M. J. G. Hunter de la Station de Biologie de l'Arctique, Office des Recherches sur les Pêcheries du Canada, Ste-Anne de Bellevue, Qué. Le premier auteur de cet article fit partie de l'expédition de 1965 et contribua à réunir les données de base de la présente étude en compagnie de M.M. I. G. Gidney et D. Patriquin. La revue de la littérature sur ce sujet a été préparée avec la collaboration de M. Vianney Legendre, Directeur du Laboratoire de Recherches au Service de la Faune du Québec. Les auteurs tiennent à exprimer leur gratitude à M. Etienne Magnin, professeur au Département de Biologie, Université de Montréal, Montréal, Qué., pour ses suggestions lors de la préparation du manuscrit. Les chiffres entre parenthèses après les noms des localités renvoient aux chiffres de la Fig. 1.

Le touladi a déjà été récolté à maintes reprises en milieu marin. On en trouve des mentions pour la baie Adlatok (1) et la baie Upaktik (2) sur la côte atlantique du Labrador (Weed, 1934), et pour les eaux saumâtres des rivières George (3) et Koksoak (4), deux tributaires de la baie d'Ungava (Dunbar et Hildebrand, 1952). Deux spécimens ont été capturés dans la baie de Castel (5) sur l'île de Banks dans les Territoires du Nord-Ouest (Manning, 1953). Un spécimen conservé à l'American Museum of Natural History a été obtenu dans la baie d'Hudson, à 5 milles au large de la côte; cette espèce a aussi été rencontrée à l'embouchure de quelques tributaires du golfe du Couronnement (6) par l'Expédition arctique canadienne de 1913–1918 (Walters, 1953). On l'a récolté en eau saumâtre à l'embouchure de la rivière Burnside (7) dans la région de Bathurst Inlet (8) (Walters, 1955), de la rivière Naknek (9) en Alaska (Rounsefell, 1958), et de la rivière Back (12) dans les Territoires du Nord-Ouest (J. G. Hunter, communication personnelle). Cependant, aucun de ces auteurs ne donne le degré de salinité à l'endroit des captures des touladis. Nos données proviennent de cinq stations: les coordonnées géographiques, les salinités, et les températures de l'eau sont indiquées dans le Tableau I.

La station 1 est dans un détroit long de 1.12 km et large de 400 m environ, partie des lacs des Eskimos (13), tributaires de la mer de Beaufort via la baie de Liverpool (14). Du 20–27 juillet 1961, sept filets maillants y ont pris 67 touladis. Les salinités minimum et maximum de l'eau de la mer durant cette

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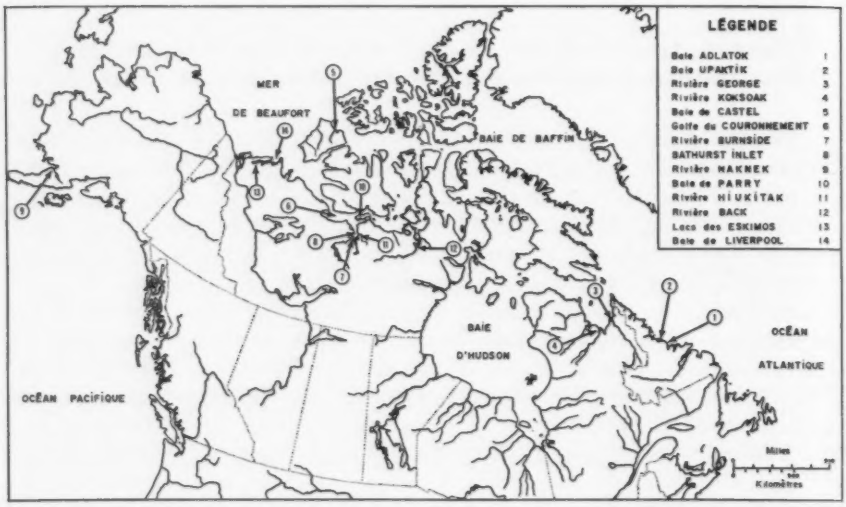

FIG. 1. Position géographique des localités nommées dans le texte. Des touladis ont été pêchés en milieux marin ou saumâtre en 1, 2, 3, 4, 5, 7, 9, 10, 11, 12 et 13.

Geographic position of the localities mentioned in the text. The grey trout were fished from either the sea or brackish water from 1, 2, 3, 4, 5, 7, 9, 10, 11, 12, and 13.

TABLEAU I. Emplacement des stations et données physiques recueillies: P = profondeur de l'eau en mètres; S = salinité de l'eau en parties par mille; Temp = température de l'eau en degrés centigrades.

Station locations and physical data recovered: P = depth of the water in meters; S = salinity of the water in parts per thousand; Temp = temperature of the water in degrees Centigrade.

Station	Lat. N.	Long. O.	No. de la Fig. 1	Date	P (*m*)	S (‰)	Temp (*C*)
1	69°13.3′	132°27.8′	13	21–7–1961	0	8.33	9.73
					5	8.41	9.75
				24–7–1961	0	7.79	10.20
				27–7–1961	0	8.15	11.50
2	69°24.5′	132°08′	13	30–7–1961	0	11.26	12.50
					10	11.60	11.02
3	69°14′	132°27′	13	8–8–1962	0	6.86	13.28
					6	6.97	13.97
4	68°18′	107°42′	10	9–8–1965	–	–	–
5	67°09′	107°18′	11	15–8–1965	0	8.6	9.4
					1.8	9.0	–
					7.3	12.8	−0.8

période de l'année était de 7.79‰ en surface et de 8.41‰ à 5 m de profondeur. A la station 2 au fond d'une baie à quelques kilomètres au nord-est de la station 1, quatre filets maillants tendus le 29 et relevés le lendemain contenaient seulement deux spécimens de *S. namaycush*. L'analyse de l'eau de mer montra ici une salinité relativement élevée: 11.26‰ en surface et 11.60‰ à 10 m de profondeur.

La station 3 était aussi située dans le détroit décrit à la station 1. Quatre filets maillants installés là le 8 août 1962, relevés le lendemain, prirent 12 touladis. Les salinités de 6.86‰ en surface, à 6.97‰ à 6 m de profondeur, accusaient alors une légère baisse sur celles du même détroit en 1961.

La station 4, à l'embouchure d'un ruisseau tributaire de la baie de Parry (10), livre le 9 août 1965 une trentaine de touladis, certains de plus de 6 kg, pris à la ligne. Ce ruisseau provient d'un lac se trouvant environ 400 m en amont, pourvu d'une population importante de touladis. Le faible débit du ruisseau ne permettait pas au moment de notre visite le passage des poissons dans un sens comme dans l'autre. Les observations semblent suggérer que ces poissons ont pu descendre à la mer avec la crue du printemps, mais que la baisse du niveau de l'eau après la fonte des neiges ne leur permit pas de retourner dans leur habitat primitif. Bien que les salinités n'aient pas été mesurées ici, l'eau à l'endroit de la capture était fort probablement saumâtre à cause de l'influence du milieu marin de la baie de Parry.

La station 5, située environ 6 km au nord-ouest de l'embouchure de la rivière Hiukitak (11), reçut le 13 août 1965, cinq filets maillants posés de la rive vers le large; ils furent relevés le lendemain et trois furent à nouveau tendus jusqu'à la journée suivante. Au total, 17 touladis furent pris: 7 mâles, 8 femelles, et 2 de sexe indéterminé, la plupart capturés dans moins de 3 m d'eau, mais quelques-uns jusqu'à 4 m de profondeur. A l'endroit de la capture, la salinité fut de 8.6‰ en surface et de 12.8‰ à une profondeur de 7.3 m, ce qui permet d'estimer une salinité se situant probablement entre 10 et 11‰ à 4 m de profondeur. De plus, deux espèces considérées comme marines ont été trouvées côte à côte avec ces touladis: *Gadus ogac* Richardson (3 spécimens) et *Eleginus sp.* (71 spécimens).

Signalons enfin que de nombreuses autres stations ont aussi été visitées dans ces mêmes régions; cependant, la salinité de l'eau dépassait 13‰ et aucun spécimen de touladi n'a été capturé à ces endroits.

Nous retiendrons deux points des faits énumérés ci-dessus. Premièrement, les mentions faites ici sur la présence du *S. namaycush* en eau saumâtre proviennent de régions côtières du Canada et de l'Alaska situées au nord du 55^{e} parallèle, ce qui limite le phénomène à la partie septentrionale de l'aire de répartition de l'espèce; cette observation rejoint d'ailleurs les opinions déjà émises à ce sujet par plusieurs auteurs (Lindsey, 1964; Rounsefell, 1958; Walters, 1955). En second lieu, vu l'abondance relative de l'espèce en eau dont la salinité varie entre 6 et 9‰, et sa rareté en eau dont la salinité varie entre 10 et 12‰, et vu son absence en eau dont la salinité dépasse 13‰, on

peut suggérer que chez *S. namaycush*, la limite supérieure de tolérance à la salinité paraît bien se situer, en nature, entre 11 et 13‰.

Département de Biologie
Université de Montréal, Montréal, Qué.

JEAN BOULVA
ANDRÉ SIMARD

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"Wet" Weight as a Method for Measuring Stomach Contents of Walleyes, *Stizostedion vitreum vitreum*

During the summer of 1966 a simple "wet" weight gravimetric procedure was used to report the stomach contents of walleyes captured by gillnets in West Blue Lake, Manitoba. Wet-weight measurements were accompanied by determinations of "dry" weight of stomach contents. The purpose of this report is to evaluate the wet-weight method using dry weight as a standard.

In the past, several methods have been used to provide quantitative information on stomach contents of walleyes. One method is to present the frequency or per cent frequency of occurrence of each kind of organism in a sample of stomachs (Eschmeyer, 1950; Houde, 1967). Koshinsky (1965) used a volumetric method to measure the relative contribution of each kind of organism to the total volume of stomach contents. A similar but gravimetric procedure was described by Le Brasseur (1966) to report the stomach contents of salmonids.

Although each method has particular advantages, frequency of occurrence methods provide less quantitative information than either volumetric or gravimetric analyses. For instance, the above enumeration methods do not provide estimates of the biomass of each diet item. On the other hand, volumetric and dry-weight gravimetric measurements of stomach contents are more difficult to make, particularly in the field, than are frequency of occurrence determinations.

Walleyes were captured in gillnets in West Blue Lake from June 19 to September 13. Stomachs from 183 fish were examined. Of this total, 76 fish had empty stomachs and 45 had less than 0.1 g (dry weight) total contents. The remaining 62 fish had 0.1 g (dry weight) or more. These fish provided data for the evaluation of the wet-weight procedure. The average fork length of the 62 fish was 377 mm and the range was from 200 to 558 mm. Their ages were from 2 to 9 years.

Stomachs and esophagi were removed from the fish, labelled, and preserved in 10% formalin. Contents were usually analysed within the week after capture. Stomachs were first rinsed with water; contents were then removed, placed on paper towels, and sorted into the following types: fish, crayfish, amphipods, insects, and miscellaneous items. Mucus and moisture were removed by blotting with paper towels. Organisms of each type were then counted, placed on tared squares of wax paper, and weighed on a trip balance. Wet weight of the total contents of each stomach was obtained by summing weights of each type present. Organisms and the squares of wax paper were placed in an oven and held at a temperature of 150 C until the samples reached a constant weight (10–16 hr, depending on the size and number of items). Dry weight of all material in a stomach was again obtained by summing weights of components.

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Comparisons of weights obtained with the trip balance with weights measured on an analytical balance indicated that wet and dry weights measured on the trip balance were accurate for weights greater than 0.1 g. Consequently only stomachs with contents weighing more than 0.1 g were included in the analyses to follow.

For all organisms commonly found in walleye stomachs, correlation coefficients, reflecting the relationship between wet and dry weights, were highly significant statistically (Table I), indicating that wet weights could be used to predict dry weights with precision. Considering the fish category, for example, the reduction in total sum of squares for dry weight was 98% (r^2 = 0.980) when dry weight was the dependent variable. Insects and miscellaneous dry weights were predicted with less precision by the appropriate wet weights (r^2 = 0.821). Approximately 18% of the total sum of squares for dry weight was not accounted for by the regression of dry weight on wet weight.

TABLE I. "Wet" and "dry" weights and correlation between "wet" and "dry" weights of different organisms present in the stomachs of walleyes captured in West Blue Lake, Manitoba, during 1966.

Category	No. of stomachs	Total weight (g)		Per cent of total		"r"	P	r^2
		Wet	Dry	Wet	Dry			
Total	62	514.59	102.82			0.990	0.01	0.980
Fish	45	463.67	95.92	90.10	93.20	0.990	0.01	0.980
Crayfish	7	23.05	2.45	4.48	2.38	0.988	0.01	0.976
Amphipods	14	16.78	2.47	3.26	2.40	0.945	0.01	0.893
Insects	6	1.25	0.36	0.24	0.35	0.906	0.05	0.821
Misc.	9	10.25	1.58	1.99	1.53	0.906	0.01	0.821

Precision may have been directly proportional to total stomach content weight. For example, fish formed the largest portion of total stomach contents (r^2 = 0.980) but insects formed less than 1% of total stomach contents (r^2 = 0.821). Another important point appeared to be that correlation between wet and dry weights was not affected by the size of the food organism. Yellow perch, *Perca fluviatilis*, which were the only fish species identified in stomach contents, ranged in weight from approximately 0.05 g wet weight to almost 80 g wet weight. Small perch fry were commonly present in walleye stomachs. Nevertheless, wet and dry weights were highly correlated (Table I). Amphipods, too, were usually individually smaller than insects, but wet weights predicted dry weights with considerable precision (r^2 = 0.893).

Results of this study indicate that the wet-weight procedure provides reliable estimates of dry weight in common situations. During field surveys wet weight is as easy if not easier to obtain than are enumeration data. Minor

constituents of stomach contents, forming less than 2% of the total should, perhaps, be regrouped to form a larger fraction or be omitted from quantitative consideration of results.

Department of Zoology C. L. GLENN
Brandon University, Brandon, Man.

Department of Zoology F. J. WARD
University of Manitoba, Winnipeg, Man.

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Cyclopecten carlottensis, a New Species of Pectinidae from the Northeastern Pacific

On October 20, 1966, five living specimens of a pectinid new to the northeastern Pacific were collected by means of an Agassiz trawl fitted with $\frac{1}{8}$-inch marquisette lining from a substrate consisting of hard clay in 1650 m of water. Faunistic associations included ophiuroids, pennatulids, and silicious sponges.

Cyclopecten carlottensis sp.nov.

Diagnosis. — Shell thin, silvery, translucent, inequivalve, convex. Dorsal margin straight. Auricles prominent. Byssal notch narrow and reduced. No ctenolium. Anterior auricle large with single radial fold, ornamented with numerous scales. Posterior auricle small, prolonged, no radial folding, ornamentation similar to anterior auricle. Left valve with 12 or more rows of recurved scales forming fragile pustules which easily erode, resulting in curved plates. Secondary rows of pustules arise towards the ventral margin. The margins of the imbrications between the pustules form narrow erect threads. Right valve the smaller, no ornamentation except for concentric plications; auricles ornamented as in left valve. Interior highly polished; pallial sinus and adductor scars very slight.

Type locality. — Fisheries Research Board Station No. 66-76, off Moresby Island, Queen Charlotte Islands, British Columbia, at 52°59′00″N lat and 133°06′00″W long. Depth 1650 m. Two more specimens were collected in 1967 at Fisheries Research Board Station No. 67-37 off Queen Charlotte Islands, British Columbia, position 53°43′00″N lat and 133°27′00″W long. Depth 1450 m.

Deposition and dimension of specimens are as follows:

	Height (*mm*)	Length (*mm*)	Diam (*mm*)	Depository
Holotype	11.5	12.7	3.1	National Museum of Canada No. 46246
Paratype A	8.0	7.5	1.5	University of British Columbia No. 7243
B	8.0	7.5	1.7	California Academy of Sciences No. 13097
C	9.0	8.0	2.0	California Academy of Sciences No. 13098
D	9.8	10.2	3.0	United States National Museum No. 678743

Comparison.— The species differs markedly from all other northeastern Pacific cyclopectens. There is a resemblance to *C. benthalis* Grau 1959, but *C. carlottensis* can be distinguished by the longer hinge line, the greater auricular proportion, and the more profuse ornamentation of the left valve. *Cyclopecten carlottensis* most closely resembles the Atlantic *C. imbrifer* (Lovèn) but lacks the pronounced concentric lines and is further distinguished by the intact pustules being covered by minute granulations. The new species differs from *C. imbrifer* specimens from the Barents Sea and Novaya Zemlya in having the pustules elongated horizontally, rather than circular or slightly longer vertically; the pustules are taller than their greatest diameter and with

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vertical sides, rather than blister-like and mucronate, and the rows of pustules are spaced more closely, being separated by approximately the horizontal width of the pustules. The margins of the imbrications between the pustules are appressed in *C. imbrifer*. The other related species, *C. pustulosus* Verrill, has a more substantial shell and the radial rows of pustules on the left valve are more widely spaced and not as high. The external appearance of *C. carlottensis* is very similar to *Propeamussium hoskynsi* (Verrill) (*non Pecten hoskynsi* G. O. Sars) but lacks the internal ribs.

Grateful thanks are extended to Dr I. McTaggart-Cowan of the University of British Columbia for kindly comparing specimens with material in the United States National Museum.

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

F. R. BERNARD

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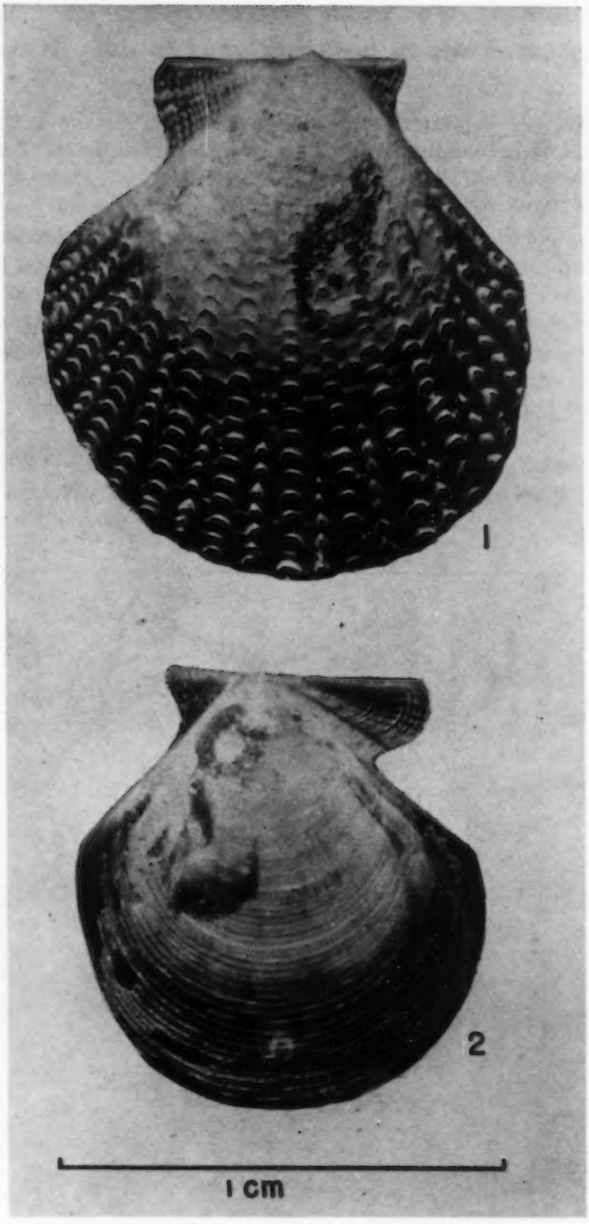

FIG. 1. *Cyclopecten carlottensis* sp.nov., right exterior view.

FIG. 2. *Cyclopecten carlottensis* sp.nov., left exterior view.

Age and Growth of Lake Sturgeon in the Saskatchewan River Delta

This study on age composition and growth rate of the lake sturgeon, *Acipenser fulvescens* Rafinesque, is based on the examination of 127 specimens collected in 4 years during the period 1954–66 from the commercial fishery at Cumberland House on the Saskatchewan River and from experimental gillnet sets made in conjunction with other studies. In Saskatchewan the species is classed as both a sport fish and a commercial species, and although it has never been of major importance to the commercial fishing industry, it has supported small but locally important commercial fisheries at Island Falls on the Churchill River and in the Saskatchewan River delta and Cumberland Lake. This study utilizes data collected in 1965 and 1966 by the senior author and material collected by J.-P. Cuerrier and F. M. Atton in 1954 and E. B. Reed in 1958.

Study area. — Within the Saskatchewan River system, lake sturgeon are found in substantial numbers and commercially exploited only in the lower reaches where the river enters the delta region (Fig. 1).

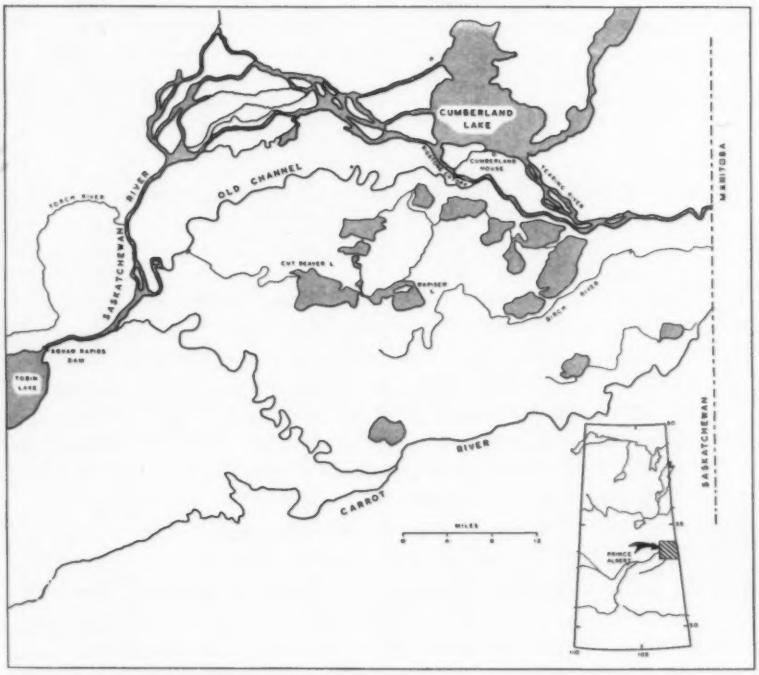

FIG. 1. The Saskatchewan River delta area. The insert shows the location of the area in Saskatchewan.

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The Saskatchewan River enters the delta a short distance downstream from the Squaw Rapids Hydro-Electric Dam. The major flow passes eastward through a low-lying area occupied by a maze of river channels and enters Cumberland Lake through three main channels on a 9-mile wide delta at the western side of the lake. Approximately 20% of the flow bypasses Cumberland Lake through the Old Channel. This flow joins the discharge from the Bigstone Cutoff and the Tearing River, and the combined flow of the Saskatchewan River flows into Cedar Lake in Manitoba and eventually into Lake Winnipeg.

The recent construction of the Squaw Rapids Dam (1962) above the delta and the Grand Rapids Dam (1964) in Manitoba has effectively isolated the sturgeon population in the Saskatchewan delta region. It is therefore of considerable interest to document the growth rates that have existed up to the present.

TABLE I. Mean lengths and weights of 127 sturgeon of different age-groups taken from the Saskatchewan River Delta during the period 1954–66.

Age-group	Frequency	Fork length (*inches*)	Weight (*lb*)	Age-group	Frequency	Fork length (*inches*)	Weight (*lb*)
1	3	11.9	0.4	26	8	44.2	22.9
2	1	19.4	1.7	27	6	47.6	30.9
3	–	–	–	28	5	47.8	27.5
4	–	–	–	29	4	48.7	29.6
5	1	27.2	3.6	30	3	47.9	29.5
6	–	–	–	31	4	48.0	30.2
7	3	28.5	4.9	32	1	48.0	32.0
8	3	29.4	5.1	33	4	51.1	37.7
9	2	29.0	5.0	34	3	48.1	37.0
10	–	–	–	35	–	–	–
11	1	33.5	7.6	36	3	52.0	38.2
12	–	–	–	37	1	49.5	36.5
13	–	–	–	38	–	–	–
14	–	–	–	39	–	–	–
15	3	37.2	15.5	40	–	–	–
16	1	41.0	16.5	41	4	52.6	42.9
17	3	40.1	17.7	42	–	–	–
18	4	43.0	22.0	43	–	–	–
19	1	42.5	20.0	44	1	49.0	35.5
20	3	43.4	19.2	45	–	–	–
21	15	42.3	20.3	46	2	55.5	42.0
22	18	44.6	23.5	47	1	54.0	43.0
23	5	44.7	21.6	50	2	57.3	54.8
24	3	45.8	25.0	51	1	52.0	34.5
25	2	45.4	26.3	54	1	55.5	48.0
				65	1	63.5	82.0

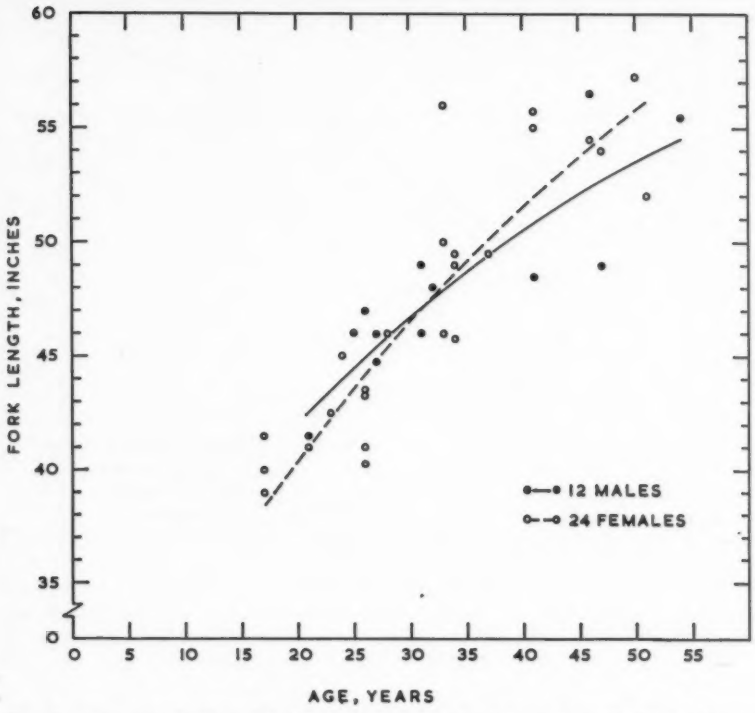

FIG. 2. Comparison of growth of male and female lake sturgeon from the Saskatchewan River delta (1954 data only).

Lake sturgeon have been commercially exploited in the delta region for various periods since about 1900. During the past decade the annual harvest has declined progressively from 7900 lb in 1958 to less than 2000 lb in 1966. The sturgeon fishery is exclusively a gillnet operation. At the present time the annual limit in the delta is set at 6000 lb, the minimum mesh size permitted is 12 inches extention measure (10-inch mesh allowed between 1962 and 1966), and the minimum legal size is 44 inches fork length.

Age and growth. — Sturgeon from the commercial fishery were routinely measured for fork length and total length, weighed in the round condition, and re-weighed after being dressed (head, fins, and viscera removed) at the time of sale. Both pectoral fins were removed and tied together with a label on which pertinent data were recorded.

In this report all unqualified length measurements refer to fork length. Conversion to total length may be made by multiplying fork length by the factor 1.0807 (average obtained from measurement of 91 specimens).

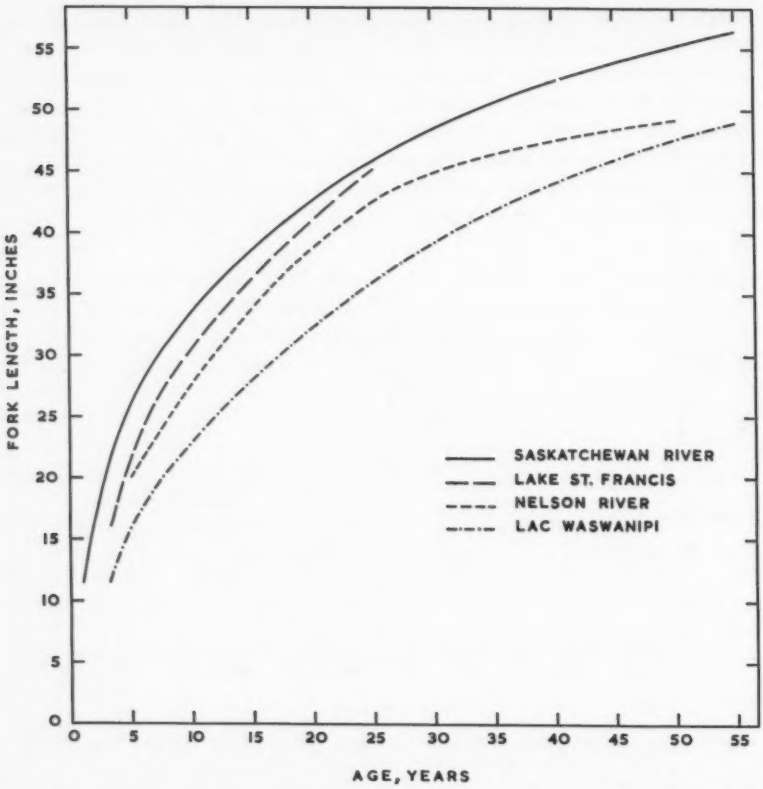

FIG. 3. Growth in length of lake sturgeon from the Saskatchewan River delta, Lake St. Francis (Cuerrier and Roussow, 1951), Nelson River (Sunde, MS, 1959), and Lac Waswanipi (Magnin, 1966). The curve for the Saskatchewan River fish was fitted by the least-squares method to data for 127 specimens (Table I).

Age determinations were made by examination of transverse sections of the marginal ray of the pectoral fin. Sections were cut from the dried bone with a motor-driven saw of a basic design described by Witt (1961). The sections were immersed in 70% alcohol and viewed under low magnification with a stereoscopic microscope. The criteria used in assigning ages to sturgeon were essentially the same as described by Cuerrier (1951).

Age-groups ranging from 1 to 65 were represented in the collections (Table I). Growth rates were compared for data collected in each of 1954, 1958, and 1965–66 but no significant differences were discerned. Sex was recorded only for the 1954 data and separate growth curves were derived by the method of least squares for males and females for that year (Fig. 2). Both lines are curvilinear. Although the sample size is very small there is a suggestion

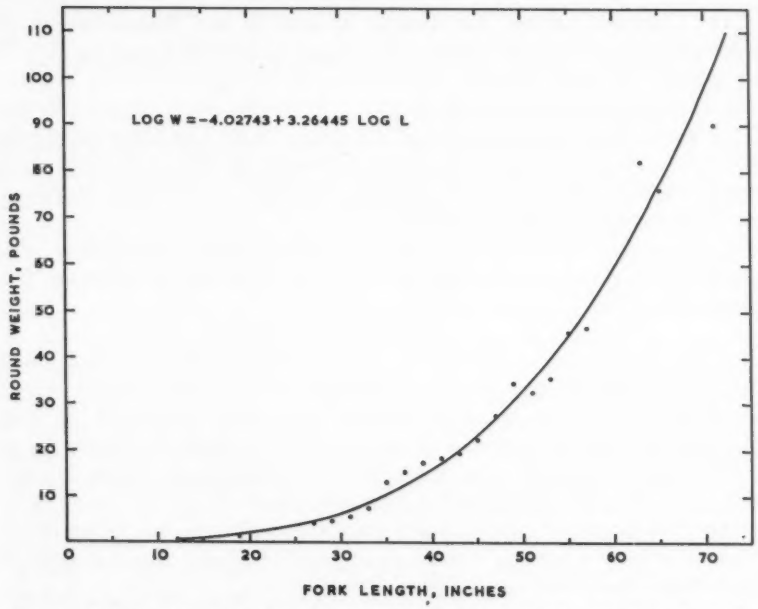

FIG. 4. Relationship of fork length to round weight for lake sturgeon from the Saskatchewan River delta. The points represent weight averaged for 2-inch length intervals; the curve is fitted by least squares method.

that males grow slightly more slowly than females at least beyond age 35. Probst and Cooper (1954) found no difference in the growth rates between the sexes in the Lake Winnebago region of Wisconsin. Sunde (MS, 1959) reports that in the Nelson River, Manitoba, growth to age 20 was similar in both sexes. However, from age 20 to 50, he reported growth of females was more rapid in both length and weight.

Because no significant differences in growth were observed between different years of sampling, and differences in the growth rates between the sexes were slight, a composite growth curve was derived by combining the data for all years of collection (Fig. 3). Growth of sturgeon in the Saskatchewan River is considerably faster than in the Nelson River and the Waswanipi Lake and River, Province of Quebec, throughout the fish's life span and slightly faster than in Lake St. Francis, Province of Québec, at least to age 25. Cuerrier (unpublished data) and Roussow (1957) found that growth of sturgeon in the Ottawa River, west of Ottawa, is also slower than in the Saskatchewan River, despite the more southern location of the former area. Sturgeon in the Saskatchewan River grow considerably slower than in the Lake Winnebago region of Wisconsin. Sturgeon from the Saskatchewan River are approximately 46.0 inches at age 20 and 56.2 inches (total lengths) at age 40; in the Lake Winnebago region, 49.8 inches and 67.0 inches total length respectively.

The average annual increments in growth for Saskatchewan River sturgeon to age 20 were 2.1 inches fork length and 0.9 lb round weight (Table I). From age 20 to 40, growth was at a rate of 0.5 inches and 1.3 lb per year. Beyond age 40, growth in length slowed to 0.3 inches per year and the average annual increment in weight increased to about 1.4 lb. The more rapid increase in weight as age advances is apparently associated with development of the gonads, which in the case of mature females may account for 21.5% of the total body weight (Cuerrier, 1966).

Length–weight relationship. — The length–weight relationship of lake sturgeon from the Saskatchewan River delta (Fig. 4) is expressed by the following formula:

$$\log W = -4.02743 + 3.26445 \log L$$

where W is round weight in pounds and L is fork length in inches. This relationship is almost identical to that reported (Probst and Cooper, 1954) for Lake Winnebago sturgeon. Thus despite differences in growth in widely separate areas, body proportions of lake sturgeon remain essentially the same.

The largest sturgeon recorded from the Saskatchewan delta during the study measured 70.0 inches fork length and weighed 110 lb (round weight). However, the largest sturgeon caught from the Saskatchewan River (province wide) was taken by an angler at Saskatoon in 1962 and was a female which measured just over 6 ft and weighed 270 lb.

Saskatchewan Fisheries Laboratory — L. M. Royer
Saskatoon, Saskatchewan — F. M. Atton

Canadian Wildlife Service — J.-P. Cuerrier
Ottawa, Ontario

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Electrophoretic Separation of Fish Brain Esterases[1]

In a previous paper (Hogan and Knowles, 1968) we described certain properties of channel catfish (*Ictalurus punctatus* (Walbaum)) and bluegill (*Lepomis macrochirus* Rafinesque) brain acetylcholinesterase (AChE). Other esterases were present in the brain homogenates, as apparent from the substrate specificity data, although the manometric technique employed did not permit their differentiation. These esterases have now been separated electrophoretically and classified by their reactions with substrates and inhibitors.

Materials and methods.— Fish brains were homogenized in an all-glass Potter–Elvehjem-type tissue grinder in 40% sucrose solution. The homogenate concentration was 10 brains/ml for both the bluegill and channel catfish. The brei was centrifuged at 34,700 *g* for 30 min at 5 C, and 30 μliters of the supernatant were used per column for electrophoresis.

Disc electrophoresis was carried out in a Canalco Model 12 apparatus (Canal Industrial Corp., Rockville, Maryland) at a constant current of 2.5 ma/column for 90 min at room temperature. Premixed chemicals were used, and the standard procedure for conducting the electrophoresis supplied with the apparatus was followed. The separating gel was 7% polyacrylamide and the buffer was tris-glycine (*p*H 8.3, $\mu = 0.01$).

On completion of electrophoresis, the gels were removed from the columns and placed in 0.5 M boric acid (*p*H 4.1) for 1 hr. After two rinses in distilled water the gels were stained for esteratic proteins. The technique of Hunter and Burstone (1960) was used; α-naphthyl acetate was the substrate and Fast Blue RR (Color Index No. 37155) was the diazonium coupler. The thiocholine method of Karnovsky and Roots (1964) as modified by Bunyan and Taylor (1966) was employed in certain experiments to detect enzymes that hydrolyze thiocholine esters. Destaining in methanol, water, and acetic acid (5:5:1) for several days yielded maximum contrast.

Inhibition of esterases was studied by placing the gels in buffered inhibitor solutions or emulsions for 30 min prior to the esterase assay. The staining solution also contained inhibitor of the same concentration as that during the inhibition reaction. The following compounds were used as potential esterase inhibitors at a concentration of 10^{-5} M: dichlorvos, 2,2-dichlorovinyl dimethyl phosphate; paraoxon, diethyl *p*-nitrophenyl phosphate; DFP, di*iso*propyl phosphorofluoridate; eserine sulfate; and *p*-chloromercuribenzoate (10^{-4} M). Esterases inhibited by both eserine and organophosphate were classified as either AChE (EC 3.1.1.7 acetycholine acetyl-hydrolase) or cholinesterase (EC 3.1.1.8 acylcholine acyl-hydrolase); those inhibited by organophosphate only were classified as carboxylesterases (EC 3.1.1.1 carboxylic

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ester hydrolase); and those not affected by either eserine or organophosphate but inhibited by *p*-chloromercuribenzoate were classified as arylesterases (EC 3.1.1.2 aryl ester hydrolase) (Augustinsson, 1961).

Results.— At least eight zones with esterolytic activity for α-naphthyl acetate were observed migrating toward the anode in the electropherogram separating soluble esterases of bluegill brain (Fig. 1). Zones 5 and 6 were the most intense, 4 and 7 were of intermediate intensity, and zones 1, 2, 3, and 8 were of low intensity. Dichlorvos, a vinyl phosphate, partially or completely inhibited all of the bluegill esterases. Paraoxon and DFP were more selective in their inhibition of bluegill esterases than was dichlorvos. The activity of zones 1, 3, 4, and 8 was reduced drastically by these compounds; however, the other zones with the possible exception of zone 7 were not apparently affected. *p*-Chloromercuribenzoate (not shown) was not inhibitory toward any of the esterase zones.

In addition to hydrolyzing α-naphthyl acetate, zone 1 also split acetylthiocholine and butyrylthiocholine; however, the former thiocholine ester was hydrolyzed to the greater extent. Eserine inhibited the hydrolysis of all three substrates. Also, an eserine-sensitive band capable of hydrolyzing α-naphthyl acetate, acetylthiocholine, and butyrylthiocholine was occasionally observed equidistant between zones 1 and 2 in certain of the gels.

Electropherograms of catfish brain showed at least six esterase zones (Fig. 2). Zones 1 and 6 were the most intense, 2 and 3 were of intermediate intensity, and zones 4 and 5 were of low intensity. Dichlorvos was the most potent inhibitor tested as it was with the bluegill esterases. Esterolytic activity of all zones was either severely reduced or absent. The inhibition pattern of paraoxon was similar to that of DFP. *p*-Chloromercuribenzoate apparently did not affect catfish esterases.

Catfish esterase zones 1, 2, and 3 (Fig. 2) also hydrolyzed acetylthiocholine and were inhibited by eserine. Zones 1 and 2 also slightly split butyrylthiocholine, and the activity was abolished by eserine. An eserine-sensitive zone that showed good activity toward acetylthiocholine but little or no activity toward butyrylthiocholine and α-naphthyl acetate was present equidistant between zones 2 and 3.

Discussion and conclusions.— Two esterase zones from the bluegill and four esterase zones from the channel catfish were "cholinesterases" since they were sensitive to inhibition by eserine at a low concentration. The fact that acetylthiocholine was hydrolyzed to a greater extent than was the *n*-butyryl analog suggested that the enzymes were of the AChE type. Moreover, in a previous study, Hogan and Knowles (1968) found that butyrylcholine was only slightly split by bluegill and channel catfish brain homogenates and that at least one enzyme with the properties of an AChE was present.

The other soluble brain esterases from both species of fish were inhibited partially or completely by the organophosphate dichlorvos; indicating that they were of the carboxylesterase (formerly aliesterase) type. It was interesting to note that the majority of the soluble fish brain esterases were not appreciably

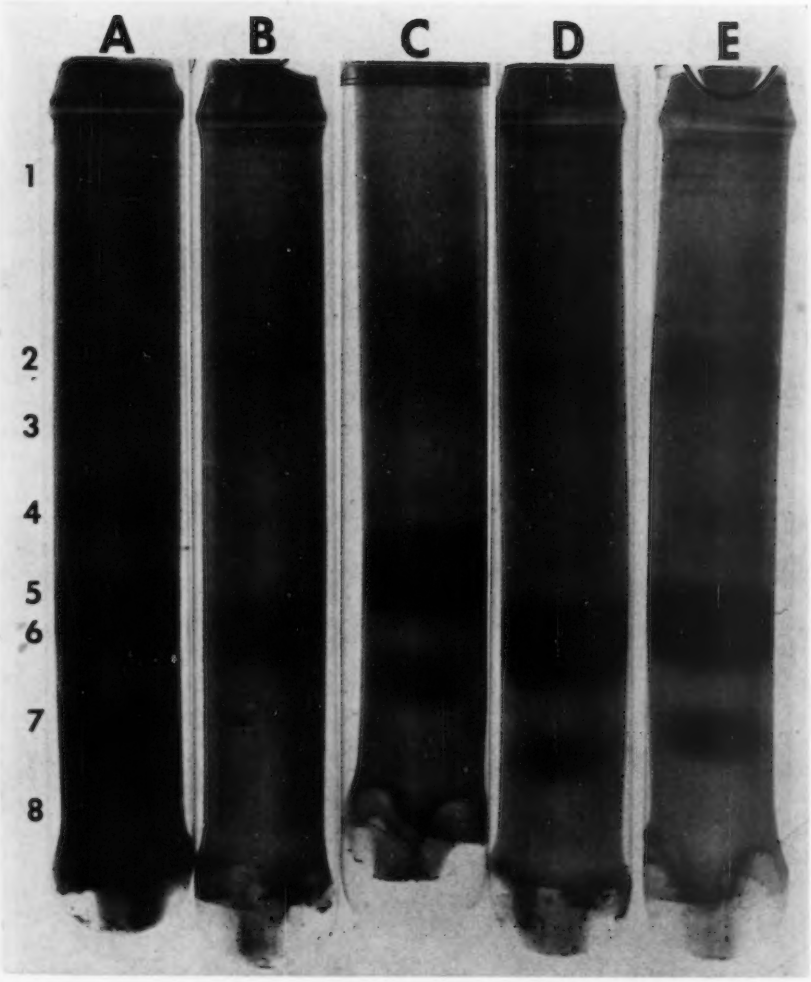

FIG. 1. Electropherograms resulting from separation of soluble esterases of bluegill brain in polyacrylamide gel. A, α-naphthyl acetate control; B, with 10^{-5} M dichlorvos; C, with 10^{-5} M paraoxon; D, with 10^{-5} M DFP; and E, with 10^{-5} M eserine. Numbers indicate zones of esterolytic activity. Origin (cathode) for electrophoresis is at the top.

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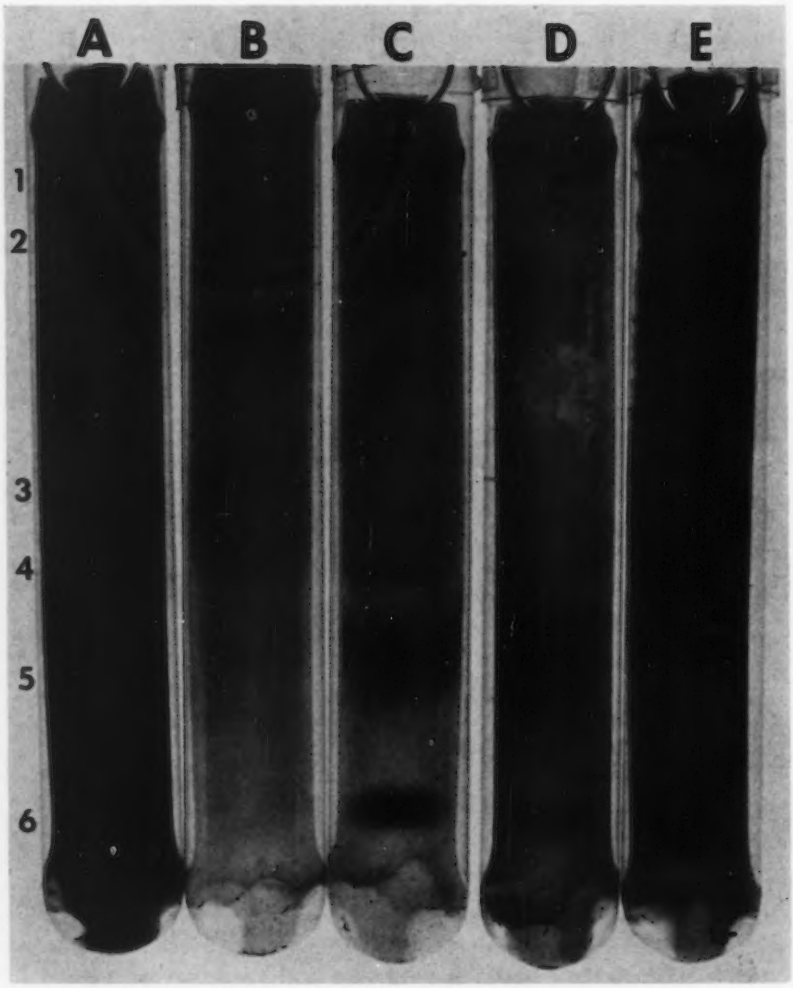

FIG. 2. Electropherograms resulting from separation of soluble esterases of channel catfish brain in polyacrylamide gel. Legend same as in Fig. 1.

affected by paraoxon or DFP at the concentration used in this investigation. However, Bernsohn et al. (1965), working with rat brain esterases, reported that the soluble enzymes were more resistant to inhibition by paraoxon than were the bound esterases.

Department of Entomology
University of Missouri, Columbia, Mo., U.S.A.

CHARLES O. KNOWLES
SURESH K. ARURKAR

Fish Pesticide Research Laboratory
U.S. Bureau of Sport Fisheries and Wildlife
Columbia, Mo.

JAMES W. HOGAN

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Aeromonas sp. Infection in Great Lakes Lampreys

In February 1967, heavy mortality occurred in ammocoetes of the American brook lamprey, *Entosphenus lamottei*, held at the University of Guelph. The ammocoetes had been previously captured in streams tributary to Lake Huron or Lake Superior within a 50-mile radius of Sault Ste. Marie, Ontario.

The dead and moribund ammocoetes exhibited haemorrhagic areas throughout their bodies, and many bled from the anus. Employing blood agar (Bacto[1] blood agar base + 8% sterile, citrated sheep blood) and an incubation temperature of 20 C, a short, Gram-negative, haemolytic, rod-shaped bacterium was isolated in pure culture from the blood of each of the five moribund and the five ammocoetes examined. Subcutaneous inoculation of this bacterium killed healthy ammocoetes of both the American brook lamprey and the sea lamprey, *Petromyzon marinus*, usually within 48 hr. Symptoms characteristic of the initial infection were evident in all the inoculated ammocoetes, and rapid decomposition of their body tissue occurred before and after death.

In February 1968, mortality occurred in ammocoetes of the sea lamprey held at Sault Ste. Marie, Ontario. These also had been taken from Lake Superior and Lake Huron streams. Moribund and dead specimens of these ammocoetes showed the same symptoms as the brook lampreys in Guelph, and the same pathogenic bacterium was isolated from them.

Intramuscular and intraperitoneal inoculations with the bacterium killed the following species of fish: *Salvelinus fontinalis*, *Salvelinus namaycush*, *Salmo gairdneri*, and *Ictalurus nebulosus*. Extensive tissue necrosis occurred at the inoculation site. The bacterium was recovered in pure culture from the heart, liver, spleen, intestine, and kidney of each inoculated fish. The same species of fish were not affected when placed in aquaria containing aerated but unchanged water heavily contaminated with the bacterium. Subcutaneous and intraperitoneal inoculations with the bacterium had no effect on white mice.

The bacterium was identified as a member of the genus *Aeromonas*. There is some uncertainty as to the taxonomy of the few species in this genus. Consequently, the bacterium infecting the ammocoetes of the brook and sea lampreys may be named *A. liquefaciens*, or *A. hydrophila*, or *A. punctata*.

This bacterial species has been associated with diseases of fish, but this is apparently the first report of its infecting and causing mortality of the ammocoetes of brook and sea lampreys. Hall (1963) noted the death of chestnut lampreys, *Ichthyomyzon castaneus*, used in aquarium experiments, from furunculosis caused by *A. salmonicida*.

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[1]Difco Laboratories, Inc., Detroit, Michigan.

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Department of Microbiology
Ontario Agricultural College
University of Guelph, Guelph, Ont.

L. A. McDERMOTT

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